GB 9515478.7

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 231 264 A1

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
14.08.2002 Bulletin 2002/33

(21) Application number: **00951548.7**

(22) Date of filing: **04.08.2000**

(51) Int Cl.[7]: **C12N 9/12**, C12N 15/54

(86) International application number:
PCT/ES00/00308

(87) International publication number:
WO 01/11027 (15.02.2001 Gazette 2001/07)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **06.08.1999 EP 99500139**

(71) Applicant: **Glaxo Wellcome, S.A.**
28760 Tres Cantos (Madrid) (ES)

(72) Inventors:
- **MOSCAT GUILLEN, Jorge,**
  Centro de Biol. Molecular
  28049 Cantoblanco (ES)
- **DIAZ-MECO CONDE, Maria Teresa**
  Centro de Bio. Mol.
  28049 Cantoblanco (ES)

(74) Representative: **Stott, Michael John**
GlaxoSmithKline
Corporate Intellectual Property (CN9.25.1)
980 Great West Road
Brentford, Middlesex TW8 9GS (GB)

(54) **NEW PROTEIN**

(57) An isolated human BLIP protein or a variant thereof.

EP 1 231 264 A1

Description

Field of the Invention

[0001] The present invention relates to human lambda interacting proteins involved in Nuclear Factor κB (NF-κB) activation and also, but not exclusively, to kinase-inactive mutants of such proteins; nucleotide sequences encoding such proteins and mutant proteins; and expression vectors, cell lines, antibodies, screening methods, compounds, methods of production and methods of treatment related to the proteins.

Background of the Invention

[0002] The atypical Protein Kinase C (PKC) isotypes (λ/ιPKC and ζPKC) are necessary for basic cellular functions such as proliferation and survival. Studies have demonstrated that the atypical PKCs (aPKCs) are stimulated by TNFα and are required for the activation of NF-κB by this cytokine, through a mechanism that likely involves the phosphorylation of IκB through the activation of IκB kinase β.

[0003] The transcription factor NF-κB plays a critical role in a number of cell functions including key inflammatory and immune responses. Nuclear factor-κB is a ubiquitously expressed multisubunit transcription factor activated in several cell types by a diverse group of inflammatory agents such as TNFα, IL-1β, bacterial endotoxin, and RNA viruses. NF-κB is composed of dimers of different members of the Rel protein family. The classical form of NF-κB is an heterodimer of p50 and p65 (Rel A) (Baeurle and Henkel, 1994; Baldwin, 1996; Thanos and Maniatis, 1995); this is sequestered in the cytosol by IκB which prevents its nuclear translocation and activity.

[0004] Upon cell stimulation by inflammatory cytokines such as TNFα or IL-1, IκBα is phosphorylated, triggering the ubiquitination and subsequent degradation of IκB through the proteosome pathway (Verma et al., 1995). These events release NF-κB which translocates to the nucleus where it activates several genes (Baeurle and Henkel, 1994; Baldwin, 1996; Thanos and Maniatis, 1995; Verma et al., 1995).

[0005] The identification of the kinase responsible for the signal-induced phosphorylation of IκB has been a matter of research. Several groups have identified and cloned two IκB kinase activities (IKKα and IKKβ) that phosphorylate residues 32 and 36 of IκBα, and whose activity is potently stimulated by TNF and IL-1 (DiDonato et al., 1997; Mercurio et al., 1997; Réginer et al., 1997; Woronicz et al., 1997; Zandi et al., 1997). The IKKs bind NIK (NFκB-inducing kinase), a member of the MAPKKK family that interacts with TRAF2 (Malinin et al., 1997), thus linking IκB degradation and NF-κB activation to the TNF receptor complex.

[0006] TNFα is a potent activator of the atypical PKCs in vivo. It has previously been shown that the atypical PKC isoforms ζ (zeta) and λ/ι (lambda/iota) play a critical role during NF-κB activation (Diaz-Meco et al., 1996; Folgueira et al., 1996; Sontag et al., 1997). Thus, the blockade of the atypical PKCs by either microinjected pseudosubstrate peptide inhibitors (Dominguez et al., 1993), antisense oligonucleotides (Folgueira et al., 1996), or the transfection of kinase-dead dominant negative mutants of ζPKC or λ/ιPKC (Berra et al., 1995; Bjorkoy et al., 1995; Diaz-Meco et al., 1996; Sontag et al., 1997), dramatically impairs NF-κB activation.

[0007] However, the mechanisms whereby the atypical PKCs participate in the NF-κB activation pathway remained unclear. Because ζPKC is unable to directly phosphorylate IκB (Diaz-Meco et al., 1994), it is possible that the signals generated by the stimulation of the atypical PKCs could be mediated by the novel IKKs. In this regard, it has recently been demonstrated that the atypical PKCs bind to the IKKs *in vitro* and *in vivo* (Lallena et al., 1999). Overexpression of these PKCs positively modulates IKKβ but not IKKα activity, whereas the transfection of their respective dominant negative mutants severely impairs the activation of IKKβ but not of IKKα in TNFα-stimulated cells (Lallena et al., 1999). In addition, recombinant active atypical PKC dramatically stimulates *in vitro* the IKKβ -- but not IKKα -- activity from unstimulated cells (Lallena et al., 1999). Collectively these results demonstrate a critical role of the atypical PKCs in the NF-κB pathway through the regulation of IKKβ activity.

[0008] In contrast to the classical or novel isoforms of PKCs, the atypical PKCs are insensitive to phorbol esters or diacyglycerol, but are activated by other important lipid second messengers such as phosphatidylinositol 3,4,5-P_3 (Nakanishi et al.,1993), and ceramide (Lozano et al., 1994). Although the products of PI 3-kinase activate the aPKCs (Nakanishi et al., 1993; Akimoto et al., 1996), they also stimulate other kinases such as εPKC, θPKC, PRK1 or c-Akt (Diaz-Meco et al., 1996). Ceramide activates both aPKCs (Lozano et al., 1994; Müller et al., 1995) but also stimulates the ceramide-activated protein kinase, among other possible targets. This suggests that the different lipid mediators are not selective for a given PKC.

[0009] It has now been discovered that a novel lambda interacting protein (termed BLIP herein) interacts with λ/ιPKC in a TNFα-dependent manner. The ectopic expression of wild-type BLIP is sufficient to activate NF-κB in a λ/ιPKC dependent manner, whereas a BLIP kinase-inactive mutant is dominant negative. Human and other mammalian BLIP proteins are useful in screening methods for the identification and development of novel pharmaceutical agents, including agonists and antagonists of the proteins, which may be used to increase or decrease the activation of NFκB

(compared to activation which would occur in an untreated control cell).

[0010] Accordingly, it is an object of the present invention to provide . isolated human BLIP proteins and DNA encoding such proteins. Other objects of the present invention will become apparent from the following detailed description thereof.

Summary of the Invention

[0011] According to one embodiment of the present invention there is provided an isolated human BLIP protein or a variant thereof. In a particularly preferred aspect of the invention the protein has an amino acid sequence as provided in **Figure 2A** (SEQ ID NO:4).

[0012] A further aspect of the present invention is a kinase-dead mutant of a BLIP protein.

[0013] A further aspect of the present invention is an isolated human LIP protein or a variant thereof. In a particularly preferred aspect of the invention the protein has an amino acid sequence of SEQ ID NO:6.

[0014] According to another aspect of the invention there is provided an isolated nucleotide sequence encoding a BLIP protein or a variant thereof, or a nucleotide sequence which is complementary thereto. Preferably the nucleotide sequence is a cDNA sequence. Particularly preferably, the nucleotide sequence is or comprises SEQ ID NO:3.

[0015] According to another aspect of the invention there is provided an isolated nucleotide sequence encoding a LIP protein or a variant thereof, or a nucleotide sequence which is complementary thereto. Preferably the nucleotide sequence is a cDNA sequence. Particularly preferably, the nucleotide sequence is or comprises SEQ ID NO:6.

[0016] According to another aspect of the invention there is provided an isolated DNA molecule having a sequence selected from the group consisting of: (a) isolated DNA which encodes the BLIP protein of **Figure 2A** (SEQ ID NO: 4); (b) isolated DNA which hybridizes to isolated DNA of (a) above under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1% SDS at 60°C, which is at least 65% homologous to the isolated DNA of (a) above, and which encodes a BLIP protein; and (c) isolated DNA having a sequence complementary to (a) - (c), above.

[0017] According to another aspect of the invention there is provided an expression vector comprising a nucleic acid sequence as referred to above. According to another aspect of the invention there is provided a nucleic acid construct having a promoter and a heterologous nucleic acid operably linked to said promoter, wherein said heterologous nucleic acid is a nucleic acid molecule as given above

[0018] According to another aspect of the invention there is provided a stable cell line comprising a recombinant DNA sequence or an expression vector as referred to above, capable of expressing the encoded a protein. Preferably the cell line is a modified HEK293 or HeLa cell line.

[0019] According to another aspect of the invention there is provided a polyclonal or monoclonal antibody that specifically binds to a BLIP protein as given above.

[0020] According to another aspect of the invention there is provided an antisense oligonucleotide complementary to a nucleic acid as given above and having a length sufficient to hybridize thereto under physiological conditions; along with DNA encoding such an antisense oligonucleotide; and a nucleic acid construct having a promoter and a heterologous nucleic acid operably linked to said promoter, wherein the heterologous nucleic acid is a DNA encoding such an antisense oligonucleotide.

[0021] According to another aspect of the invention there is provided a method of inhibiting NF-κB activation in a cell which comprises providing to the cell an inhibitor of BLIP-related NF-κB activation, in an amount effective to inhibit NF-κB activation therein. The cell may be provided in any suitable form, such as in in vitro culture. The providing step may be carried out by any suitable means, such as by delivering the inhibitory compound into the cell or by delivering into the cell a nucleic acid encoding and expressing an inhibitory protein. The cell is preferably a mammalian cell and more preferably a human cell.

[0022] A further aspect of the present invention is the use of a BLIP kinase-dead mutant, or a LIP protein, as an inhibitor of NF-κB activation in a cell, or as an anti-cancer agent or adjuvant.

[0023] According to another aspect of the invention there is provided a method for screening compounds for the ability to modulate (enhance or inhibit) BLIP-dependent activation of NF-κB. Such methods comprise contacting a test compound with a BLIP protein as referred to above and detecting modulating activity or inactivity.

[0024] According to another aspect of the invention there is provided a compound that modulates BLIP -dependent NF-κB activity, identifiable by the methods referred to above.

[0025] According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder that is responsive to modulation of BLIP-dependent NF-κB activation, in a mammalian subject, that comprises administering to said patient an effective amount of a compound that modulates (inhibits or enhances) BLIP-dependent NF-κB activation. Preferably the disorder is selected from neoplastic, inflammatory and immune disorders.

[0026] According to a further aspect of the invention there is provided use of a compound that modulates of BLIP-dependent NF-κB activation in a method of formulating a medicament for treatment or prophylaxis of a disorder that

is responsive to modulation of BLIP-dependent NF-κB activation.

Brief Description of the Figures

[0027] The present invention will be further described by way of example and with reference to the following figures:

Figure 1 graphs the effect of overexpression of LIP on NF-κB activation; increasing expression of LIP decreased TNFα-induced κB-dependent reporter activity.

Figure 2A shows the amino acid sequence of the human BLIP protein (SEQ ID NO:4).

Figure 2B is a schematic representation of BLIP showing the FRAP/ATM PI 3-kinase-like domain and the LIP region.

Figure 3A is an alignment of the PI 3-kinase domain of human BLIP with that of FRAP. Asterisks indicate amino acids that are identical in both aligned proteins; the conserved critical residues that confer catalytic activity to the protein are boxed.

Figure 3B is an alignment of the PI 3-kinase domain of human BLIP with that of ATM. Asterisks indicate amino acids that are identical in both aligned proteins; the conserved critical residues that confer catalytic activity to the protein are boxed.

Figure 4A graphs the effects of BLIP on NP-κB activation in cultures of human HEK293 cells. The HEK293 cells were transfected with a κB-dependent luciferase reporter plasmid and either: a negative control vector (first bar); increasing concentrations of an expression plasmid for wild-type BLIP (BLIP; second - sixth bars); increasing concentrations of an expression plasmid for kinase-inactive BLIP ($BLIP^{MUT}$; seventh - eleventh bars); or an RIP expression vector (positive control; final bar). After 24 hours the levels of luciferase expression were determined. Results are the mean ± SD of three independent experiments with incubations in duplicate.

Figure 4B graphs the role of BLIP on NF-κB activation in cultures of HEK293 cells. Cells were transfected with a κB-dependent luciferase reporter plasmid and either: a negative control vector (white bars), an expression plasmid for wild type λ/ιPKC (λ/ιPKC; hatched bars), or an expression plasmid for kinase-inactive λ/ιPKC ($\lambda/\iota PKC^{MUT}$; solid bars). Luciferase activity in cells without BLIP (control) and with increasing concentrations of wild-type BLIP (BLIP) was assessed after 24 hours. Results are the mean ± SD of three independent experiments with incubations in duplicate.

Figure 5 shows the percentage of HeLa cells with nuclear p65 after transfection with a vector for an HA-tagged version of $BLIP^{MUT}$. Twenty-hours post-transfection, cells were either untreated (control; white bars) or stimulated for 20 min with TNFα (30 ng/ml; solid bars). Analysis was by immunofluorescence confocal microscopy with monoclonal anti-HA antibody 12CA5 and a goat polyclonal anti-p65 antibody. The graph shows the mean ± SD of several fields (300 cells) from three independent experiments.

Figure 6 provides the nucleotide sequence (SEQ ID NO:3) of DNA encoding the BLIP protein of SEQ ID NO:4.

Detailed Description of the Invention

[0028] The present inventors have isolated and characterized a novel LIP isoform, herein termed BLIP (for "big LIP") (SEQ ID NO:4). BLIP interacts with λ/ιPKC in a TNFα-dependent manner, and activates NF-κB in a λ/ιPKC-dependent manner. Of functional relevance, BLIP has a domain that is highly homologous to the PI 3-kinase-like region of FKBP-rapamycin associated protein (FRAP) and Ataxia Telangiectasia Mutated (ATM) protein (see **Figs. 3A** and **3B**). Interestingly, the ectopic expression of wild-type BLIP is sufficient to activate NF-κB in a λ/ιPKC dependent manner, whereas a BLIP kinase-inactive mutant is dominant negative. This is the first evidence of the involvement of a FRAP/ATM-like molecule in the activation of NF-κB. NF-κB has been shown to be relevant in cell proliferation and apoptosis (Baldwin, 1996; Lenardo and Baltimore, 1989). The present inventors further expanded the known sequence of the human LIP protein, and determined that the LIP protein inhibits NF-κB dependent transcription when expressed in a cell.

[0029] The atypical PKCs are regulated by protein regulators. Compared to the amino acid sequences of classical or novel PKCs, the sequences of the atypical PKC subfamily differ in the regulatory domain. The identification of selective modulators for each PKC isotype will assist in understanding the function and regulation of the different PKC

isoforms. A protein named LIP has been identified as a selective regulator of λ/ιPKC (Diaz-Meco et al., 1996). LIP binds to the zinc finger of λ/ιPKC but does not bind to αPKC, εPKC or even to the highly related ζPKC (Diaz-Meco et al., 1996). Interestingly, overexpression of a fragment of LIP (the R4 fragment) that is sufficient to interact with λ/ιPKC is capable of stimulating a κB-dependent promoter activity (Diaz-Meco et al., 1996).

[0030] Previously it was reported that expression of a minimal C-terminal region of LIP (named R4 in Diaz-Meco et al., 1996) that bound λ/ιPKC was also sufficient to activate NF-κB (Diaz-Meco et al., 1996). However, the present inventors determined that the overexpression of full-length LIP does not activate NF-κB, and in fact inhibits κB-dependent gene transcription (see Example 4).

[0031] LIP was initially identified by the two-hybrid system in yeast. An isolated cDNA of LIP encoding a protein of 713 amino acids with a predicted molecular weight of 79.7 kDa is described by Diaz-Meco et al. (1996). The present inventors determined the sequence of LIP to be 779 amino acids (SEQ ID NO:6), and observed that LIP was expressed in testicle and heart tissues but not in other tissues investigated (liver, ovary, spleen, thymus, brain, lung and kidney). However, a band that reacted with the anti-LIP antibody and having a molecular weight higher than 200 kDa was unexpectedly detected (results not shown).

[0032] The expression of LIP and the newly identified band was then determined in two different human cell lines (HEK293 and HeLa cells). See Example 2. It was observed that both bands were detectable in both cell lines although the proportion varied, with the high molecular weight protein (BLIP) more abundant in HEK293 than in HeLa cells (results not shown).

[0033] The present inventors assessed whether BLIP could bind to λ/ιPKC in vivo (Example 3), using HEK293 cells that were either untreated (control) or stimulated with TNFα for various times. Under resting conditions there was little or no association of BLIP or LIP to λ/ιPKC (data not shown). However, upon TNFα stimulation there was a rapid and sustained interaction of BLIP with λ/ιPKC.

[0034] To further investigate whether BLIP was physiologically relevant in the aPKC/NF-κB pathway, the present inventors next cloned and molecularly characterized BLIP (Example 5). BLIP is a protein of 2392 amino acids (**Fig. 2A**, SEQ ID NO:4); the region encompassing amino acids 881-1209 of BLIP displays a high degree of homology with the PI 3-kinase domains of FRAP (SEQ ID NO:1) and ATM (SEQ ID NO:2) (**Figs. 3A** and **3B**). The alignment of the PI 3-kinase-like domains of BLIP, FRAP and ATM indicates that BLIP has kinase activity, as the DXXXXN and DFG motifs that constitute critical residues of the catalytic site conserved in all ATM family members with kinase activity, are also conserved in BLIP (see **Figs. 3A** and **3B**).

[0035] The present inventors investigated whether the FRAP/ATM-like kinase domain of BLIP plays a role in NF-κB activation (Example 6). HEK293 cells were transfected with a κB-dependent reporter gene (luciferase) along with increasing concentrations of expression plasmids for BLIP, either wild-type BLIP or a kinase-inactive mutant ($BLIP^{MUT}$). The results (**Figure 4A**) indicate that the expression of wild-type BLIP (but not a kinase inactive mutant BLIP) is sufficient to activate the κB-dependent reporter activity to an extent comparable to that of RIP (Receptor Interacting Protein).

[0036] Further experiments established that the NF-κB activation effect of BLIP is dependent on λ/ιPKC (Example 7). Cells were transfected with a κB-dependent luciferase reporter plasmid and either a negative control vector, an expression plasmid for wild type λ/ιPKC, or an expression plasmid for kinase-inactive $\lambda/\iota PKC^{MUT}$. Luciferase activity in cells without BLIP (control) and with increasing concentrations of wild-type BLIP (BLIP) was assessed, and it was seen that the co-expression of wild-type λ/ιPKC cooperated with BLIP to activate NF-κB, whereas the expression of the kinase-inactive λ/ιPKC mutant did not. Results are shown in **Figure 4B**.

[0037] The present inventors' work indicates that BLIP is a critical component of the NF-κB pathway, and depends on its own kinase activity and that of λ/ιPKC to exert its function.

[0038] To demonstrate that BLIP controls the translocation of RelA to the nucleus, cells were transfected with the HA-tagged BLIP mutant expression vector ($BLIP^{MUT}$). Twenty-four hours post-transfection the cells were either stimulated with TNFα or untreated (control). The cells were then analyzed by double immunofluorescence using an anti p65 (RelA) antibody and anti HA-antibody to detect cells that were expressing the construct (data not shown). Analysis of the cells revealed cells transfected with the BLIP mutant had inhibited TNFα-induced translocation of p65 compared to cells transfected with wild-type BLIP **(Fig. 5)**.

[0039] The atypical PKCs are involved in the control of NF-κB and p70S6K (Romanelli et al., 1999). Both pathways are critical in the control of inflammatory and immunosuppressor cell function. NF-κB controls a myriad of immune responses and is a potent anti-apoptotic molecule. Inhibition of the atypical PKC kinases blocks the growth and proliferation of cells and initiates a programmed cell death (Berra et al., 1993, 1997; Diaz-Meco et al., 1996; Murray et al., 1997). In addition, the inhibition of aPKCs leads to a reversion of the transforming phenotype of Ras-transformed cells (Bjorkoy et al., 1995), indicating that inhibitors of the atypical PKCs may have anti-neoplastic effects and would be suitable for screening for anti-cancer therapeutic effects. Consistent with this notion, the atypical PKCs target key intermediaries in the cell signaling cascades involved in cell proliferation. It has been demonstrated that ζPKC as well as λ/ιPKC control the activation of the MEK-ERK signaling cascade, along with Raf, in the Ras and the PI 3-kinase γ pathways (Diaz-Meco et al., 1994, 1995; Liao et al., 1997; Schönwasser et al., 1998; Takeda et al., 1999).

[0040] The present inventors determined that BLIP has a domain highly homologous to the kinase region of FRAP. FRAP is the target of the immunosupressor molecule rapamycin (Dennis et al., 1999). Rapamycin blocks the activation of p70S6K in T cells which constitutes the molecular basis of its immunosuppressor actions (Dennis et al., 1999). Interestingly, the activity of p70S6K is required and sufficient for the cell cycle progression of T lymphocytes. The BLIP-λ/ιPKC tandem represents an alternate pathway to that of FRAP in the design of potentially novel immunosuppressor molecules.

[0041] The results reported herein demonstrate the critical role of BLIP in NF-κB activation, and also demonstrate that the FRAP/ATM-like kinase domain of BLIP is important in NF-κB activation. A kinase-dead mutant of BLIP protein is unable to activate NF-κB and even has a dominant negative activity when transfected in TNFα-activated cells (similar to the effects of the full-length LIP).

[0042] While not wishing to be held to a single theory underlying the mechanisms of the LIP and BLIP proteins, the present investigators postulate that the full-length LIP constrains the ability of λ/ιPKC to activate NF-κB. This constraint is not present when the R4 fragment is used to investigate the activation of NF-κB (Diaz-Meco et al., 1996). It is possible that in the case of BLIP, the putative constraint is relieved by the action of the FRAP/ATM-like kinase activity.

[0043] Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

[0044] Amino acid sequences disclosed herein are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence. Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with established usage.

[0045] As referred to above, the present invention relates to isolated BLIP and LIP proteins. Sequence information for isolated human BLIP is provided in **Figure 2A** (amino acid; SEQ ID NO:4) and **Fig. 6** (nucleotide; SEQ ID NO:3). Amino acid sequence information for a kinase-inactive mutant of BLIP is provided in SEQ ID NO:5. Sequence information for LIP is provided in SEQ ID NO:6 (amino acid) and SEQ ID NO:7 (nucleotide). In the context of this invention the term "isolated" indicates that the protein or nucleotide molecule is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods. The term "isolated" therefore includes the possibility of the molecule being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, expression vectors, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the protein is in a state as found in nature.

[0046] Routine methods known in the art, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the proteins and nucleotide molecules according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook, J. et al (1), the disclosure of which is included herein in its entirety by way of reference.

[0047] The term "BLIP variant" as used throughout the specification and claims refers to other peptides or proteins that retain the same essential functional character of the BLIP protein of SEQ ID NO:4 provided herein, but which vary in amino acid sequence. Variants are also intended to be included within the scope of the invention. For example, peptides or proteins with greater than about 65%, preferably at least 70%, 75%, 80% or 85%, and particularly preferably at least about 90% or even 95% or 98% sequence similarity with SEQ ID NO:4, and which retain BLIP functions, are considered as variants of the BLIP protein. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the basic biological functionality of a BLIP protein, i.e., interacts with λ/ιPKC in a TNFα-dependent manner and activates NF-κB in a TNFα-dependent and λ/ιPKC-dependent manner. This biological functionality can be assessed by conducting studies as described herein and as known in the art.

[0048] The term "BLIP protein" as used herein does not include a protein consisting only of the LIP protein sequence (SEQ ID NO:6) or the R4 fragment (SEQ ID NO:8).

[0049] As used herein, a BLIP protein is one having amino acid SEQ ID NO:4, or having amino acid similarity of at least about 65%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or even 99% to SEQ ID NO:4 and having BLIP functions. A protein having BLIP functions is one that interacts with λ/ιPKC in a TNFα-dependent manner, activates NF-κB in a TNFα-dependent and λ/ιPKC -dependent manner, and contains a functional kinase domain. The degree of amino acid sequence similarity between proteins can be calculated using various commercially available computer software programs (e.g., BLAST analysis, as is known in the art).

[0050] As used herein, a kinase inactive mutant of BLIP is a protein or peptide having substantial sequence similarity to a functional BLIP protein (e.g., sequence similarity of at least about 65%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or even 99%), but having a non-functional kinase domain. One skilled in the art will recognize the PI-3 kinase domain

of a BLIP protein by its sequence. An example of a kinase-inactive BLIP mutant is provided herein by SEQ ID NO:5.

[0051] The term "LIP variant" as used throughout the specification and claims refers to other peptides or proteins that retain the same essential functional character of the LIP protein of SEQ ID NO:6 provided herein, but which vary in amino acid sequence. Variants are also intended to be included within the scope of the invention. For example, peptides or proteins with greater than about 90%, 92%, 95% or even 97% or 98% sequence similarity with SEQ ID NO:6, and which retain LIP functions, are considered as variants of the LIP protein. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the basic biological functionality of a LIP protein, i.e., inhibits NF-κB dependent transcription. This biological functionality can be assessed by conducting studies as described herein and as known in the art. As used herein, a LIP protein is one having amino acid SEQ ID NO:6, or having amino acid similarity of at least about 90%, 92%, 95%, 97%, 98% or even 99% to SEQ ID NO:6 and having LIP functions. The degree of amino acid sequence similarity between proteins can be calculated using various commercially available computer software programs (e.g., BLAST analysis, as is known in the art).

[0052] In general, those skilled in the art will appreciate that minor deletions or substitutions may be made to the amino acid sequences of peptides and proteins of the present invention without unduly adversely affecting the activity thereof. Thus, proteins containing such deletions or substitutions are a further aspect of the present invention. In peptides containing substitutions or replacements of amino acids, one or more amino acids of a sequence may be replaced by one or more other amino acids wherein such replacement does not affect the function of that sequence. Such changes can be guided by known similarities between amino acids in physical features such as charge density, hydrophobicity/hydrophilicity, size and configuration, so that amino acids are substituted with other amino acids having essentially the same functional properties, as is known in the art.

[0053] The invention also includes nucleotide sequences that encode BLIP and LIP proteins, mutants, and variants thereof as well as nucleotide sequences which are complementary thereto. The nucleotide sequence may be RNA or DNA including genomic DNA, synthetic DNA or cDNA. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. (Nucleotide sequence information is provided in **Figure 6** (SEQ ID NO:3); the BLIP protein of SEQ ID NO:4 is encoded by nucleotides 152 - 7327 of SEQ ID NO:3). Nucleotide molecules can be isolated from human cells or synthesised according to methods known in the art, as described by way of example in Sambrook, J. et al (1), the disclosure of which is included herein in its entirety by reference. The nucleotide molecules according to the invention have utility in production of the proteins according to the invention, which may take place *in vitro, in vivo* or *ex vivo*. The nucleotide molecules may be involed in recombinant protein synthesis or indeed as therapeutic agents in their own right, utilised in gene therapy techniques. Nucleotides complementary to those encoding BLIP proteins, or antisense sequences, may also be used in gene therapy, such as in strategies for down-regulation of expression of the proteins of the invention.

[0054] As used herein, DNA molecules that encode a BLIP protein is intended to encompass natural allelic variations in the DNA molecules. Hybridization conditions which permit other DNA sequences which code for a BLIP protein to hybridize to a DNA sequence as given herein (e.g., SEQ ID NO:3) are, in general, high stringency conditions. For example, hybridization of such sequences may be carried out under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, 0.1% SDS at 60°C or even 70°C in a standard in situ hybridization assay. See J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory)). In general, DNA sequences which code for a BLIP protein and hybridize to a DNA sequence of SEQ ID NO:3 disclosed herein will have at least 65%, 70%, 75%, 80%, 85%, 90%, 92% or even 95% nucleotide sequence similarity with SEQ ID NO: 3, and encode a protein have BLIP functions. Sequence similarity may be determined using commercially available computer software programs (e.g., BLAST analysis as is known in the art). Isolated DNA of the present invention can be of any species of origin, including mouse, rat, rabbit, cat, porcine, and human, but is preferably of mammalian origin and most preferably of human origin.

[0055] As used herein, a DNA molecule encoding a LIP protein is intended to encompass natural allelic variations in the DNA molecules. Hybridization conditions which permit other DNA sequences which code for a LIP protein to hybridize to a DNA sequence as given herein (e.g., SEQ ID NO:7) are, in general, high stringency conditions. For example, hybridization of such sequences may be carried out under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, 0.1% SDS at 60°C or even 70°C in a standard in situ hybridization assay. See J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory)). In general, DNA sequences which code for a LIP protein and hybridize to a DNA sequence of SEQ ID NO:3 disclosed herein will have at least 65%, 70%, 75%, 80%, 85%, 90%, 92% or even 95% nucleotide sequence similarity with SEQ ID NO:3, and encode a protein have LIP functions. Sequence similarity may be determined using commercially available computer software programs (e.g., BLAST analysis as is known in the art). Isolated DNA of the present invention can be of any species of origin, including mouse, rat, rabbit, cat, porcine, and human, but is preferably of mammalian origin and most preferably of human origin.

[0056] Further, oligonucleotide molecules that code for the BLIP or LIP proteins (or mutants or variants thereof), but

which differ in codon sequence from the foregoing DNA molecules due to the degeneracy of the genetic code, are also an aspect of this invention. The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is well known in the art. Oligonucleotide molecules that code for the same BLIP protein as coded for by the foregoing sequences, but which differ in codon sequence from these due to site directed mutigenesis are yet another aspect of this invention. Site directed mutagenesis techniques are known in the art.

[0057] The present invention also includes expression vectors that comprise nucleotide sequences encoding the present proteins, mutants or variants thereof. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation to allow protein expression. Suitable vectors will be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook et al (1), the disclosure of which is included herein in its entirety.

[0058] Vectors may be used either to amplify DNA encoding a desired protein and/or to express DNA which encodes a desired protein. The DNA sequence encoding the desired protein is operably linked to suitable control sequences capable of effecting the expression of the protein in a suitable host. DNA regions are operably linked or operably associated when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Vectors may include plasmids, viruses (e.g., adenovirus, cytomegalovirus), phage, and integratable DNA fragments (i.e., fragments integratable into the host genome by recombination).

[0059] The invention also includes cell lines that have been modified or transfected to express the proteins of the present invention. Such cell lines include transient, or preferably stable higher eukaryotic cell lines (such as mammalian cells or insect cells), lower eukaryotic cells (such as yeast) or prokaryotic cells (such as bacterial cells). It is also possible for the proteins of the invention to be transiently expressed in a cell line, such as for example in a baculovirus expression system.

[0060] According to another aspect, the present invention also relates to antibodies (polyclonal or preferably monoclonal antibodies) that specifically bind to the proteins of the present invention (i.e., antibodies which bind to a single antigenic site or epitope on the proteins). Such antibodies are preferably of monoclonal origin. The antibodies are preferably IgG antibodies of any suitable species, such as rat, rabbit, or horse, but are generally of mammalian origin. Fragments of IgG antibodies which retain the ability to specifically bind the proteins of the present invention, such as $F(ab')_2$, F(ab'), and Fab fragments, are intended to be encompassed by the term "antibody" herein. See generally E. Harlow and D. Lane, Antibodies: A Laboratory Manual (1988)(New York: Cold Spring Harbor Laboratory Press). The antibodies may be chimeric, as described by M. Walker et al., Molecular Immunol. 26, 403 (1989). Antibodies that bind to BLIP proteins, but not to shorter LIP proteins, are particularly preferred.

[0061] Such antibodies are useful in purification, isolation or screening involving immunoprecipitation techniques and may be used as tools to further elucidate the BLIP protein function, or as therapeutic agents in their own right.

[0062] Nucleic acid oligodeoxynucleotides compementary to and capable of hybridizing to the sense strand of a gene, or to an mRNA transcribed from that gene, are antisense oligodeoxynucleotides. When the sense strand of a target gene, or an mRNA, is exposed to its antisense oligodeoxynucleotides, interaction of the two will occur such that the gene will be blocked from transcription or the mRNA blocked from translation. The present invention includes oligomers capable of hybridizing to a portion of a gene (or an mRNA) encoding the BLIP or LIP protein. When such an oligomer is hybridized to the target molecule, production of the protein will be substantially prevented or reduced (compared to that which would occur in the absence of the antisense oligonucleotide). Substantial prevention means that the protein is not produced, or is produced at non-functional levels.

[0063] Antisense oligonucleotides (and nucleic acids that express such antisense molecules) may be made in accordance with conventional techniques. See, e.g., U.S. Pat. No. 5,023,243 to Tullis; U.S. Pat. No. 5,149,797 to Pederson et al. The length of the antisense oligonucleotide (i.e., the number of nucleotides therein) is not critical so long as it binds selectively to the intended location; this can be determined in accordance with routine procedures. In general, the antisense oligonucleotide will be from 8, 10 or 12 nucleotides in length up to 20, 30, or 50 nucleotides in length. Such antisense oligonucleotides may be oligonucleotides wherein at least one, or all, of the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For example, every other one of the internucleotide bridging phosphate residues may be modified as described. In another nonlimiting example, such antisense oligonucleotides are oligonucleotides wherein at least one, or all, of the nucleotides contain a 2' loweralkyl moiety (e.g., C1-C4, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-propenyl, and isopropyl). For example, every other one of the nucleotides may be modified as described. See also P. Furdon et al., Nucleic Acids Res. 17, 9193-9204 (1989); S. Agrawal et al., Proc. Natl. Acad. Sci. USA 87, 1401-1405 (1990); C. Baker et al., Nucleic Acids Res. 18, 3537-3543 (1990); B. Sproat et al., Nucleic Acids Res. 17, 3373-3386 (1989); R. Walder

and J. Walder, Proc. Natl. Acad. Sci. USA 85, 5011-5015 (1988).

[0064] A further aspect of the present invention is screening methods designed to identify compounds that modulate BLIP activation of λ/ιPKC, or that modulate NF-κB activation. By the term "modulation" it is meant that there will be either an increase or a decrease in NF-κB activation in the presence of the compound (compared to that which would be observed in the absence of the compound); such activity generally will result from ligand binding of the compound to BLIP, λ/ιPKC, the tandem of BLIP and λ/ιPKC, or to NF-κB itself.

[0065] In general terms, such screening methods involves administering a test compound to a cell and detecting BLIP-related activation of NF-κB, compared to a control cell in which the test compound (or functional BLIP protein) is absent. Activation of NF-κB may be assessed using a κB-dependent reporter gene (such as luciferase), or by measuring translocation of NF-κB to the cell nucleus (using methods that are known in the art, e.g., the use of anti-Rel65 antibody to detect nuclear NF-κB). Such screening methods may further comprise detecting whether said test compound binds to λ/ιPKC (or BLIP, or NF-κB), e.g., by preparing cell extracts and immunoprecipitating the protein of interest, and immunoblotting using an antibody directed to the test compound.

[0066] The ability of compounds to modulate BLIP autophosphorylation activity can also be used as a method of screening compounds for NF-κB activation or inhibitory effects. In general terms, such screening methods involves administering a test compound to a cell and detecting BLIP autophosphorylation, compared to a control cell in which the test compound is absent.

[0067] The present invention also includes within its scope those compounds that are identified as possessing useful NF-κB modulation activity, by the screening methods referred to above.

[0068] Another aspect of the present invention is the use of compounds that inhibit or enhance NF-κB activation (including compounds identified by the screening techniques referred to above), in the treatment or prophylaxis of disorders which are responsive to modulation of BLIP activity or modulation of NF-κB activity, in a mammalian (preferably human) patient. The present invention thus provides a method of inhibiting (or enhacing) NF-κB activation in a cell, by providing to the cell a compound that inhibits (or enhances) BLIP-dependent NF-κB activation. An example of such an inhibitory compound is a kinase dead BLIP mutant (e.g., a protein of SEQ ID NO:5), or an antisense nucleotide as described above, where the inhibitory compound is given in an amount effective to decrease endogenous BLIP-dependent NF-κB activation in the cell (compared to NF-κB activation that would occur in the cell in the absence of the inhibitory compound). Compounds that enhance NF-κB activation are provided in an amount effective to increase endogenous BLIP-dependent NF-κB activation in the cell (compared to NF-κB activation that would occur in the cell in the absence of the compound). The providing step may be carried out by any suitable means, such as by delivering the inhibitory compound into the cell, or by delivering into the cell a nucleic acid encoding and expressing the inhibitory compound.

[0069] Compounds that inhibit the BLIP-related activation of NF-κB will have therapeutic benefit in pathologies where such activation is upregulated. In many cells of the immune system NF-κB is activated in response to various pathogenic signals, and induces transcription of a variety of genes encoding immunologically relevant proteins. Baeuerle and Henkel, *Annu Rev. Immunol.* 12:141 (1994). Interference with the activation or activity of NF-κB may suppress immunological reactions, including that seen in toxic/septic shock, graft-vs-host reactions, acute inflammatory reactions and radiation damage. In cancerous or neoplastic cells, both chemotherapy and radiation therapy can activate NF-κB, which in turn reduces the apoptotic effects of the therapy. Inhibition of NF-κB activation in tumors has been reported to enhance the apoptotic potential of cancer treatments. Wang et al., *Nature Medicine* 5:412 (1999).

[0070] Accordingly, BLIP-mediated NF-κB activation is implicated in immunological and neoplastic disease. Therefore, inhibition of BLIP-mediated NF-κB activation will provide a positive therapeutic benefit where immune suppression is desired, or where anti-neoplastic effects are desired. In particular, such inhibitory compounds will have utility for treatment and/or prophylaxis of disorders such as toxic/septic shock, graft-vs-host reactions, acute inflammatory reactions and radiation damage. Such compounds will further have use as anti-neoplastic agents in the treatment of tumors or cancers, and as adjuvants in the chemotherapy or radiation therapy of neoplastic growths. For example, adenoviral delivery to cancer cells of a kinase-dead mutant of BLIP (or another inhibitor of BLIP-dependent NF-κB activation), will be useful as an adjuvant in the chemotherapy of cancers, to increase the apoptotic effects of the chemotherapies.

[0071] Compounds that increase or enhance the BLIP-related activation of NF-κB will have therapeutic benefit in pathologies where such activation is reduced, or where it is desired to upregulate NF-κB activation. Such compounds are useful as immunostimulators and as inhibitors of cell death.

[0072] NF-κB modulating compounds, including those identified according to the screening methods outlined above, may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art, and as fully described in Remmington's Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed, 1985, the disclosure of which is included herein in its entirety by way of reference. The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

[0073] The present invention will now be further described, by way of example, in the appended experimental section.

Example 1

Materials and Methods

Reagents and Cell Cultures:

[0074] Recombinant human TNFα was purchased from Promega; monoclonal 12CA5 anti-Hemagglutinin (anti-HA) antibody from Boehringer; monoclonal antibody against λPKC from Transduction Laboratories; goat polyclonal anti-p65 (NF-κB) antibody from Santa Cruz Biotechnologies, Inc. The rabbit affinity-purified anti-LIP has been described (Díaz-Meco et al., 1996). Human HEK293 and HeLa cells were obtained from the American Type Culture Collection (ATCC). Cultures of HEK293 cells were maintained in high glucose Dulbecco's modified Eagle's medium containing 10% fetal calf serum, penicillin G (100 μg/ml), and streptomycin (100 μg/ml) (Flow). HeLa cells were maintained in minimum essential medium Eagle supplemented with 0.1 mM non-essential amino acids, 1.0 mM sodium piruvate and 10% fetal calf serum. Subconfluent cells were transfected by the calcium phosphate method (Clontech Laboratories, Inc.).

Plasmids:

[0075] To clone the cDNA for BLIP coding sequence, a human brain Marathon Ready cDNA (Clontech Laboratories, Inc.) was used following the manufacturer's procedures. This cDNA was subcloned into the NotI site of the pCDNA3-HA vector to make the pCDNA3-HA-BLIP construct. pCDNA3-HA-BLIP^{MUT} (D1066A; N1071K) was obtained by site directed mutagenesis (Quick Change, Stratagene). pCDNA3-HA-λ/ιPKC and pCDNA3-HA-λ/ιPKC^{MUT} have previously been described (Díaz-Meco et al., 1996).

Immunoprecipitations:

[0076] For immunoprecipitations of endogenous proteins, HEKl293 cells were used. Some cells were stimulated with 30 ng/ml of TNF-α. Cells were then harvested and lysed in buffer PD [40 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% Nonidet P-40, 6 mM EDTA, 6 mM EGTA, 10 mM β-glycerophosphate, 10 mM NaF, 10 mM PNPP, 300 μM Na_3VO_4, 1mM benzamidine, 2 M PMSF, aprotinin 10 μg/ml, leupeptin 1μg/ml, pepstatin 1 μg/ml, 1mM DTT], and lmg of whole-cell lysate was diluted in PD buffer and incubated with 10 μg of the monoclonal anti-λ/ιPKC antibody. This reaction mixture was incubated on ice for 2 hours, and then 25 μl of protein A or G beads was added and the mixture was left to incubate with gentle rotation for an additional 1 hour at 4°C. The immunoprecipates were then washed three times with PD buffer. Samples were fractionated on 8% SDS-PAGE, transferred to Nitrocellulose ECL membrane (Amersham) and subjected to Western blot analysis with anti-LIP. Proteins were detected with the ECL reagent (Amersham).

Luciferase reporter assays:

[0077] Subconfluent cultures of HEK293 cells were transfected with different amounts of plasmid pCDNA3-HA-BLIP or pCDNA3-HA-BLIP^{MUT} together with the κB-Luc reporter plasmid either with or without expression vectors for wild type and dominant negative λ/ιPKC(pCDNA3-HA-λ/ιPKC and pCDNA3-HA-λ/ιPKC^{MUT}). After 24 hours, cells were stimulated with TNF-α for 4 hours. Extracts were prepared and luciferase activity determined as described (Díaz-Meco et al., 1996).

Immunofluorescence:

[0078] HeLa cells were grown on glass coverslips in growth media. Subconfluent cells were transfected with 5 μg of HA-BLIP^{MUT}. Twenty-hours post-transfection, cells were stimulated with TNF-α (30 ng/ml) for 20 min. Cells were rapidly washed twice in ice-cold PBS, and fixed in 4% formaldehyde for 15 minutes at room temperature. Cells were washed four times with PBS and permeabilized with 0.1 % Triton X-100. Free aldehyde groups were quenched with 50 mM NH4Cl. Endogenous peroxidase activity was quenched by treatment with 1% H_2O_2 in PBS for 15 minutes. The fixed cells were blocked in blocking solution. Cells were incubated with the different antibodies for 1 hour at 37°C. Transfected HA-tagged protein was visualized with the monoclonal 12CA5 anti-HA (Boehringer Mannheim), and an FITC-conjugated anti-mouse (Cappel), and the endogenous p65 with a goat polyclonal antibody (Santa Cruz Biotech), and the tetramethylrhodamine tyramide TSA-Direct amplification system (NEN Life Science Products). Glass coverslips were mounted on Mowiol and were examined with an MRC 1024 Bio-Rad confocal system (Bio-Rad Richmond, CA) mounted

on a Zeiss Axiovert 135 microscope (Zeiss, Oberkochen, Germany).

Example 2

Detection of BLIP

[0079] In the course of a series of immunoblot analysis to determine the expression of LIP in different murine organs and tissues, the present inventors observed that LIP was expressed in testicle and heart tissues but not in other tissues investigated (liver, ovary, spleen, thymus, brain, lung and kidney). However, a band that reacted with the anti-LIP antibody and having a molecular weight higher than 200 kDa was unexpectedly detected (results not shown).

[0080] The expression of LIP and the newly identified band was then determined in two different human cell lines routinely used in the laboratory (HEK293 cells and HeLa cells). It was observed that both bands were detectable in both cell lines although the proportion varied, with the high molecular weight protein more abundant in HEK293 than in HeLa cells (results not shown).

Example 3

Binding of BLIP to λ/ιPKC

[0081] The present inventors assessed whether BLIP could bind to λ/ιPKC in vivo. Using HEK293 cells, the cells were either untreated (control) or stimulated with TNFα for various times, after which cell extracts were prepared, immunoprecipitated with a selective anti-λ/ιPKC antibody, and analyzed by immunoblotting with the anti-LIP antibody (results not shown). Under resting conditions there was little or no association of BLIP (or LIP; also not shown) to λ/ιPKC. However, upon TNFα stimulation there was a rapid and sustained interaction of BLIP with λ/ιPKC. This indicates that BLIP displays a stimulus-induced association with λ/ιPKC.

Example 4

LIP Inhibits NF-κB Dependent Transcription

[0082] To determine the effect of over-expressing LIP (SEQ ID NO:6) on NF-κB activation, HEK293 cells were transfected with a κB-dependent reporter gene (luciferase) along with increasing concentrations of an expression plasmid for LIP (0.2, 0.5, 1.0 and 2.0 μg/60mm diameter well). Twenty-hour post-transfection cells were either untreated or stimulated with 30 ng/ml TNFα. The results (mean ± SD of three independent experiments with incubation in duplicate; **Figure 1**) demonstrate that the expression of LIP decreases TNFα-induced κB-dependent reporter activity.

Example 5

Cloning of BLIP

[0083] The present inventors next cloned and molecularly characterized human BLIP. The BLIP clone (SEQ ID NO: 3) expanded the LIP sequence previously reported by Diaz-Meco et al. (1996). BLIP was found to be a protein of 2392 amino acids (**Fig. 2A**, SEQ ID NO:4) with a predicted molecular weight of 269 kDa. The region encompassing amino acids 881-1209 of BLIP displays a high degree of homology with the PI 3-kinase domains of FRAP and ATM (**Figs. 3A** and **3B**). The alignment of the PI 3-kinase-like domains of BLIP, FRAP and ATM indicates that BLIP has kinase activity, as the DXXXXN and DFG motifs that constitute critical residues of the catalytic site conserved in all ATM family members with kinase activity, are also conserved in BLIP. In contrast to FRAP and ATM, however, the kinase region of BLIP is not located at the most C-terminal part of the molecule but roughly in the middle (**Fig. 2B**). The C-terminus portion of BLIP is the sequence corresponding to LIP. Therefore, LIP constitutes a domain of BLIP (**Fig. 2B**), revealing a domain organization that differs from other PI 3-kinases. Upstream of the PI 3-kinase domain of FRAP there are a series of protein-protein interaction modules called the HEAT domains (Dennis et al., 1999). Such domains (or other regions through which one could predict interactions with other proteins) are absent in the BLIP molecule, with the exception of the LIP region whereby λ/ιPKC interacts (Dennis et al., 1999). Another feature of FRAP that is absent in LIP is the FRB domain; this domain is the region responsible for the interaction of FRAP with the FKB/rapamycin complex (Dennis et al., 1999). This suggests that BLIP is rapamycin-insensitive.

Example 6

Kinase Domain of BLIP

[0084] To investigate whether the FRAP/ATM-like kinase domain of BLIP plays a role in NF-κB activation, HEK293 cells were transfected with a κB-dependent reporter gene along with increasing concentrations (0.2, 0.5, 1.0, 2.0 μg/60mm diameter well) of expression plasmids for BLIP (either wild-type (SEQ ID NO:4) or a kinase-inactive mutant (SEQ ID NO:5)). As a positive control, cells were also transfected with a RIP (Receptor Interacting Protein) expression vector (RIP is a potent activator of NF-κB). The results (**Figure 4A**) indicate that the expression of wild-type BLIP (but not mutant BLIP) is sufficient to activate the κB-dependent reporter activity to an extent comparable to that of RIP.

Example 7

λ/ιPKC and BLIP-related NF-κB activation

[0085] To determine whether the NF-κB activation effect of BLIP is dependent on λ/ιPKC, HEK293 cells were transfected with a κB-dependent luciferase reporter plasmid and one of the following: a negative control vector, an expression plasmid for wild type λ/ιPKC, or an expression plasmid for kinase-inactive λ/ιPKC. Luciferase activity in cells without BLIP (control) and with increasing concentrations (0.5, 1.0, 2.0 μg/60mm diameter) of wild-type BLIP (BLIP) was assessed after 24 hours. Results are shown in **Figure 4B**. The co-expression of wild-type λ/ιPKC cooperates with BLIP to activate NF-κB, whereas the expression of the kinase-inactive λ/ιPKC mutant abrogates this effect of BLIP.

Example 8

Translocation of RelA to the Cell Nucleus

[0086] To further demonstrate that BLIP controls the actual translocation of RelA to the nucleus, HeLa cells were transfected with the HA-tagged BLIP mutant expression vector ($BLIP^{MUT}$); as control cells, non-transfected HeLa cells were used. Twenty-four hours post-transfection the cells were either stimulated with TNFα for 20 minutes or untreated (control). The cells were then analyzed by double immunofluorescence using an anti p65 (RelA) antibody and anti HA-antibody to detect cells that were expressing the construct (data not shown). Analysis of the cells indicates that more than of the 80% of cells transfected with wildtype BLIP mutant had TNFα-induced translocation of p65, compared to about 20% of cells transfected with BLIP mutant (**Figure 5**).

Example 9

Screening Methods

[0087] Using an appropriate mammalian cell line (such as HEK293 or HeLa cells), a test compound is administered to the test cells; no test compound is administered to control cells. The cells are then stimulated with TNFα for various times, after which cell extracts are prepared, immunoprecipitated with a selective anti-λ/ιPKC antibody, and analyzed by immunoblotting with the anti-BLIP antibody. An increase in the association of BLIP with λ/ιPKC in the test cells (compared to the association found in control cells) indicates that the test compound enhances BLIP-dependent λ/ιPKC activation (and thus NF-κB activation); a decrease indicates the test compound inhibits BLIP-dependent λ/ιPKC activation (and thus NF-κB activation).

[0088] Alternatively, the cells may be transfected with a κB-dependent reporter gene, such as luciferase, and the activity of the reporter gene assessed as a measure of NF-κB activation in the presence of a test compound, compared to control cells.

References

[0089]

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: a Laboratory Manual. 2nd Edition. CSH Laboratory Press. (1989)

2. Akimoto, K., K. Mizuno, S. Osada, S. Hirai, S. Tanuma, K. Suzuki, and S. Ohno. 1994. A new member of the third class in the Protein Kinase C Family, PKC, expressed dominantly in an undifferentiated mouse embryonal

carcinoma cell line and also in many tissues and cells. J. Biol. Chem. 269: 12677-12683.

3. Baeuerle, P.A., and T. Henkel. 1994. Function and activation of NF-κB in the immune system. Annu. Rev. Immunol. 12: 141-179.

4. Baldwin, A.S. 1996. The NF-κB and IκB proteins: new discoveries and insights. Annu. Rev. Immunol. 14: 649-683.

5. Berghe, W.V., S. Plaisance, E. Boone, K. De Bosscher, M.L. Schmitz, W. Fiers, and G. Haegeman. 1998. p38 and the extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-κB p65 transactivation mediated by tumor necrosis factor. J. Biol. Chem. 273: 3285-3290.

6. Berra, E., M.T. Diaz-Meco, J. Lozano, S. Frutos, M.M. Municio, P. Sanchez, L. Sanz, and J. Moscat. 1995. Evidence for a role of MEK and MAPK during signal transduction by protein kinase C ζ. EMBO J. 14: 6157-6163.

7. Bjorkoy, G., A. Overvatn, M.T. Díaz-Meco, J. Moscat, and T. Johansen. 1995. Evidence for a bifurcation of the mitogenic signaling pathway activated by ras and phosphatidylcholine-hydrolyzing. J. Biol. Chem. 270: 21299-21306.

8. Dennis, PB., S. Fumagalli, and G. Thomas. 1999. Target of rapamacyn (TOR): balancing the opposite forces of protein synthesis and degradation. Curr. Opin. Gen. & Devel. 9: 49-54.

9. Diaz-Meco, M.T., E. Berra, M.M. Municio, L. Sanz, J. Lozano, V. Díaz-Golpe, M.T. Lain de Lera, J. Alcamí, C. V. Payá, F. Arenzana-Seisdedos, J.L. Virelizier, and J. Moscat. 1993. A dominant negative protein kinase C ζ subspecies blocks NF-κB activation. Mol.Cell.Biol. 13: 4770-4775.

10. Díaz-Meco, M.T., J. Lozano, M.M. Municio, E. Berra, S. Frutos, L. Sanz, and J. Moscat. 1994. ζPKC Induces Phosphorylation and Inactivation of IκB-α In Vitro. EMBO J. 13: 2842-2848.

11. Diaz-Meco, M.T., J. Lozano, M.M. Municio, E. Berra, S. Frutos, L. Sanz, and J. Moscat. 1994. Evidence for the in vitro and in vivo interaction of Ras with protein kinase C ζ. J. Biol. Chem. 269: 31706-31710.

12. Diaz-Meco, M.T., M.M. Municio, P. Sanchez, J. Lozano, and J. Moscat. 1996. Lambda-interacting protein, a novel protein that specifically interacts with the zinc finger domain of the atypical protein kinase C isotype λ/ι and stimulates its kinase activity *in vitro* and *in vivo*. Mol. Cell. Biol. 16:105-114.

13. DiDonato, J.A., M. Hayakawa, D.M. Rothwarf, E. Zandi, and M. Karin. 1997. A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. Nature 388: 548-554.

14. Dominguez, I., L. Sanz, F. Arenzana-Seisdedos, M.T. Díaz-Meco, J.L. Virelizier, and J. Moscat. 1993. Inhibition of protein kinase ζ subspecies blocks the activation of a NF-κB-like activity in *Xenopus laevis* oocytes. Mol.Cell. Biol. 13:1290-1295.

15. Folgueira, L., J.A. McElhinny, G.D. Bren, W.S. MacMorran, M.T. Díaz-Meco, J. Moscat, and C.V. Payá. 1996. Protein kinase C-ζ mediates NF-κB activation in human immunodeficiency virus-infected monocytes. J. Virol. 70: 223-231.

16. Jung, M., Y. Zhang, S. Lee and A. Dritschilo. 1995. Correction of radiation sensitivity in ataxia telangiectasia cells by a truncated I kappa B-alpha. Science 268:1619-21.

17. Lallena, M. J., M.T. Diaz-Meco, G. Bren, C.V. Payá, and J. Moscat. 1999. Activation of IκB Kinase b by Protein Kinase C Isoforms. Mol. Cell. Biol. 19: 2180-2188.

18. Lenardo, M., and D. Baltimore. 1989. NF-κB: a pleitropic mediator of inducible and tissue-specific gene control. Cell 58: 227-229.

19. Liao, D.F., B. Monia, N. Dean, and B.C. Berk. 1997. Protein kinase C-ζ mediates angiotensin II activation of ERK1/2 in vascular smooth muscle cells. J. Biol. Chem. 272: 6146-6150.

20. Lozano, J., E. Berra, M.M. Municio, M.T. Diaz-Meco, I.Dominguez, L. Sanz, and J. Moscat. 1994. Protein kinase C ζ isoform is critical for κB-dependent promoter activation by sphingomyelinase. J. Biol. Chem. 269:19200-19202.

21. Malinin, N.L., M.P. Boldin, A.V. Kovalenko, and D. Wallach. 1997. MAP3K-related kinase involved in NF-κB induction by TNF, CD95 and IL-1. Nature 385: 540-544.

22. Mercurio, F., H. Zhu, B.W. Murray, A. Shevchenko, B.L. Bennett, J.W. Li, D.B. Young, M. Barbosa, M. Mann, A. Manning, and A. Rao. 1997. IKK-1 and IKK-2: cytokine-activated IκB kinases essential for NF-κB activation. Science 278: 860-866.

23. Müller, G., M. Ayoub, P. Storz, J. Rennecke, D. Fabbro, and K. Pfizenmaier. 1995. PKCζ is a molecular switch in signal transduction of TNFα, bifunctionally regulated by ceramide and arachidonic acid. EMBO J. 14: 1961-1969.

24. Murray, N.R. and A.P. Fields. 1997. Atypical protein kinase C iota protects human leukemia cells against drug-induced apoptosis. J. Biol. Chem. 272: 27521-27524.

25. Nakanishi, H., K.A. Brewer, and J.H. Exton. (1993). Activation of the isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate J. Biol. Chem. 268: 13-16.

26. Réginer, C.H., H.Y. Song, X. Gao, D.V. Goeddel, Z. Cao, and M. Rothe. 1997. Identification and characterization of an IκB kinase. Cell 90: 373-383.

27. Romanelli, A. K. A. Martin, A. Toker, and J. Blenis. 1999. P70 S6 kinase is regulated by protein kinase c zeta and participates in a phosphoinositide 3-kinase-regulated signalling complex. Mol. Cell Biol. 19:2921-8.

28. Rzymkiewicz, D.M., T. Tetsuka, D. Daphna-Iken, S. Srivastava, and A.R. Morrison. 1996. Interleukin-1β activates protein kinase C ζ in renal mesangial cells. J. Biol. Chem. 271: 17241-17246.

29. Schönwasser, D.C., R.M. Marais, C.J. Marshall, and P.J. Parker. 1998. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. Mol. Cell. Biol. 18: 790-798.

30. Sontag, E., J.M. Sontag, and A. Garcia. 1997. Protein phosphatase 2A is a critical regulator of protein kinase C ζ signaling targeted by SV40 small t to promote cell growth and NF-κB activation. EMBO J. 16: 5662-5671.

31. Stancovski, I., and D. Baltimore. 1997. NF-κB activation: The IκB kinase revealed? Cell 91: 299-302.

32. Takeda, H., T. Matozaki, T. Takada, T. Noguchi, T. Yamao, M. Tsuda, F. Ochi, K. Fukunaga, K. Inagaki, and M. Kasuga. 1999. PI 3-kinase gamma and protein kinase C-ζ mediate RAS-independent activation of MAP kinase by a Gi protein-coupled receptor. EMBO J 18:386-395.

33. Thanos, D., and T. Maniatis. 1995. NF-κB: a lesson in family values. Cell 80: 529-532.

34. Verma, I.M., J.K. Stevenson, E.M. Schwarz, D. VanAntwerp, and S. Miyamoto. 1995. Rel/NF-κB/IκB family: intimate tales of association and dissociation. Genes Dev. 9: 2723-2735.

35. Woronicz, J.D., X. Gao, Z. Cao, M. Rothe and D.V. Goeddel. 1997. IκB kinase-β: NF-KB activation and complex formation with IκB kinase-α and NIK. Science 278: 866-869.

36. Zandi, E., D.M. Rothwarf, M. Delhase, M. Hayakawa, and M. Karin. 1997. The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. Cell 91: 243-252.

SEQUENCE LISTING

<110> Glaxo Wellcome, S.A.

<120> Protein Kinase C Interacting Protein Involved in NF-KB Activation

<130> PI 3718

<140>
<141>

<160> 8

<170> PatentIn Ver. 2.0

<210> 1
<211> 309
<212> PRT
<213> Homo sapiens

<400> 1

```
Leu Glu Leu Gln Tyr Val Ser Pro Lys Leu Leu Met Cys Arg Asp Leu
  1               5                  10                  15

Glu Leu Ala Val Pro Gly Thr Tyr Asp Pro Asn Gln Pro Ile Ile Arg
             20                  25                  30

Ile Gln Ser Ile Ala Pro Ser Leu Gln Val Ile Thr Ser Lys Gln Arg
         35                  40                  45

Pro Arg Lys Leu Thr Leu Met Gly Ser Asn Gly His Glu Phe Val Phe
     50                  55                  60

Leu Leu Lys Gly His Glu Asp Leu Arg Gln Asp Glu Arg Val Met Gln
 65                  70                  75                  80

Leu Phe Gly Leu Val Asn Thr Leu Leu Ala Asn Asp Pro Thr Ser Leu
                 85                  90                  95

Arg Lys Asn Leu Ser Ile Gln Arg Tyr Ala Val Ile Pro Leu Ser Thr
            100                 105                 110

Asn Ser Gly Leu Ile Gly Trp Val Pro His Cys Asp Thr Leu His Ala
        115                 120                 125

Leu Ile Arg Asp Tyr Arg Glu Lys Lys Lys Ile Leu Leu Asn Ile Glu
    130                 135                 140
```

```
His Arg Ile Met Leu Arg Met Ala Pro Asp Tyr Asp His Leu Thr Leu
145                 150                 155                 160

Met Gln Lys Val Glu Val Phe Glu His Ala Val Asn Asn Thr Ala Gly
                165                 170                 175

Asp Asp Leu Ala Lys Leu Leu Trp Leu Lys Ser Pro Ser Ser Glu Val
            180                 185                 190

Trp Phe Asp Arg Arg Thr Asn Tyr Thr Arg Ser Leu Ala Val Met Ser
        195                 200                 205

Met Val Gly Tyr Ile Leu Gly Leu Gly Asp Arg His Pro Ser Asn Leu
    210                 215                 220

Met Leu Asp Arg Leu Ser Gly Lys Ile Leu His Ile Asp Phe Gly Asp
225                 230                 235                 240

Cys Phe Glu Val Ala Met Thr Arg Glu Lys Phe Pro Glu Lys Ile Pro
                245                 250                 255

Phe Arg Leu Thr Arg Met Leu Thr Asn Ala Met Glu Val Thr Gly Leu
            260                 265                 270

Asp Gly Asn Tyr Arg Ile Thr Cys His Thr Val Met Glu Val Leu Arg
        275                 280                 285

Glu His Lys Asp Ser Val Met Ala Val Leu Glu Ala Phe Val Tyr Asp
    290                 295                 300

Pro Leu Leu Asn Trp
305
```

<210> 2
<211> 120
<212> PRT
<213> Homo sapiens

<400> 2

```
Pro Ala Ile Trp Phe Glu Lys Arg Leu Ala Tyr Thr Arg Ser Val Ala
  1               5                  10                  15

Thr Ser Ser Ile Val Gly Tyr Ile Leu Gly Leu Gly Asp Arg His Val
             20                  25                  30

Gln Asn Ile Leu Ile Asn Glu Gln Ser Ala Glu Leu Val His Ile Asp
         35                  40                  45
```

```
Leu Gly Val Ala Phe Glu Gln Gly Lys Ile Leu Pro Thr Pro Glu Thr
     50                  55                  60

Val Pro Phe Arg Leu Thr Arg Asp Ile Val Asp Gly Met Gly Ile Thr
 65                  70                  75                  80

Gly Val Glu Gly Val Phe Arg Arg Cys Cys Glu Lys Thr Met Glu Val
                 85                  90                  95

Met Arg Asn Ser Gln Glu Thr Leu Leu Thr Ile Val Glu Val Leu Leu
            100                 105                 110

Tyr Asp Pro Leu Phe Asp Trp Thr
        115                 120
```

<210> 3
<211> 9138
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (152)..(7327)

<400> 3

```
agaaaaaata gagtttcact ctgttgccca ggctggagtg cagtgacgca atctcggctc 60

actgcagcct ctgccctctg agttcaagtg attctcctgc ctcagcctcc tgagtagctg 120

ggattacagg tgtctgccac cgtgcccaga c atg aaa aaa ctg ctt cct aac    172
                                   Met Lys Lys Leu Leu Pro Asn
                                     1               5

atg tta agt ccg gat ccg agg gaa ctt cag aaa tcc att gaa gtt caa   220
Met Leu Ser Pro Asp Pro Arg Glu Leu Gln Lys Ser Ile Glu Val Gln
        10                  15                  20

ttg tta aga agt tct gtt tgt ttg gca act gct tta aac ccg ata gaa   268
Leu Leu Arg Ser Ser Val Cys Leu Ala Thr Ala Leu Asn Pro Ile Glu
    25                  30                  35

caa gat cag aag tgg cag tct ata act gaa aat gtg gta aag tac ttg   316
Gln Asp Gln Lys Trp Gln Ser Ile Thr Glu Asn Val Val Lys Tyr Leu
40                  45                  50                  55

aag caa aca tcc cgc atc gct att gga cct ctg aga ctt tct act tta   364
Lys Gln Thr Ser Arg Ile Ala Ile Gly Pro Leu Arg Leu Ser Thr Leu
                60                  65                  70
```

```
aca gtt tca cag tct ttg cca gtt cta agt acc ttg cag ctg tat tgc   412
Thr Val Ser Gln Ser Leu Pro Val Leu Ser Thr Leu Gln Leu Tyr Cys
            75                  80                  85

tca tct gct ttg gag aac aca gtt tct aac aga ctt tca aca gag gac   460
Ser Ser Ala Leu Glu Asn Thr Val Ser Asn Arg Leu Ser Thr Glu Asp
        90                  95                  100

tgt ctt att cca ctc ttc agt gaa gct tta cgt tca tgt aaa cag cat   508
Cys Leu Ile Pro Leu Phe Ser Glu Ala Leu Arg Ser Cys Lys Gln His
    105                 110                 115

gac gtg agg cca tgg atg cag gca tta agg tat act atg tac cag aat   556
Asp Val Arg Pro Trp Met Gln Ala Leu Arg Tyr Thr Met Tyr Gln Asn
120                 125                 130                 135

cag ttg ttg gag aaa att aaa gaa caa aca gtc cca att aga agc cat   604
Gln Leu Leu Glu Lys Ile Lys Glu Gln Thr Val Pro Ile Arg Ser His
                140                 145                 150

ctc atg gaa tta ggt cta aca gca gca aaa ttt gct aga aaa cga ggg   652
Leu Met Glu Leu Gly Leu Thr Ala Ala Lys Phe Ala Arg Lys Arg Gly
            155                 160                 165

aat gtg tcc ctt gca aca aga ctg ctg gca cag tgc agt gaa gtt cag   700
Asn Val Ser Leu Ala Thr Arg Leu Leu Ala Gln Cys Ser Glu Val Gln
        170                 175                 180

ctg gga aag acc acc act gca cag gat tta gtc caa cat ttt aaa aaa   748
Leu Gly Lys Thr Thr Thr Ala Gln Asp Leu Val Gln His Phe Lys Lys
    185                 190                 195

cta tca acc caa ggt caa gtg gat gaa aaa tgg ggg ccc gaa ctt gat   796
Leu Ser Thr Gln Gly Gln Val Asp Glu Lys Trp Gly Pro Glu Leu Asp
200                 205                 210                 215

att gaa aaa acc aaa ttg ctt tat aca gca ggc cag tca aca cat gca   844
Ile Glu Lys Thr Lys Leu Leu Tyr Thr Ala Gly Gln Ser Thr His Ala
                220                 225                 230

atg gaa atg ttg agt tct tgt gcc ata tct ttc tgc aag tct gtg aaa   892
Met Glu Met Leu Ser Ser Cys Ala Ile Ser Phe Cys Lys Ser Val Lys
            235                 240                 245

gct gaa tat gca gtt gct aaa tca att ctg aca ctg gct aaa tgg atc   940
Ala Glu Tyr Ala Val Ala Lys Ser Ile Leu Thr Leu Ala Lys Trp Ile
        250                 255                 260
```

```
cag gca gaa tgg aaa gag att tca gga cag ctg aaa cag gtt tac aga   988
Gln Ala Glu Trp Lys Glu Ile Ser Gly Gln Leu Lys Gln Val Tyr Arg
    265                 270                 275

gct cag cac caa cag aac ttc aca ggt ctt tct act ttg tct aaa aac   1036
Ala Gln His Gln Gln Asn Phe Thr Gly Leu Ser Thr Leu Ser Lys Asn
280                 285                 290                 295

ata ctc act cta ata gaa ctg cca tct gtt aat acg atg gaa gaa gag   1084
Ile Leu Thr Leu Ile Glu Leu Pro Ser Val Asn Thr Met Glu Glu Glu
                300                 305                 310

tat cct cgg atc gag agt gaa tct aca gtg cat att gga gtt gga gaa   1132
Tyr Pro Arg Ile Glu Ser Glu Ser Thr Val His Ile Gly Val Gly Glu
            315                 320                 325

cct gac ttc att ttg gga cag ttg tat cac ctg tct tca gta cag gca   1180
Pro Asp Phe Ile Leu Gly Gln Leu Tyr His Leu Ser Ser Val Gln Ala
        330                 335                 340

cct gaa gta gcc aaa tct tgg gca gcg ttg gcc agc tgg gct tat agg   1228
Pro Glu Val Ala Lys Ser Trp Ala Ala Leu Ala Ser Trp Ala Tyr Arg
    345                 350                 355

tgg ggc aga aag gtg gtt gac aat gcc agt cag gga gaa ggt gtt cgt   1276
Trp Gly Arg Lys Val Val Asp Asn Ala Ser Gln Gly Glu Gly Val Arg
360                 365                 370                 375

ctg ctg cct aga gaa aaa tct gaa gtt cag aat cta ctt cca gac act   1324
Leu Leu Pro Arg Glu Lys Ser Glu Val Gln Asn Leu Leu Pro Asp Thr
                380                 385                 390

ata act gag gaa gag aaa gag aga ata tat ggt att ctt gga cag gct   1372
Ile Thr Glu Glu Glu Lys Glu Arg Ile Tyr Gly Ile Leu Gly Gln Ala
            395                 400                 405

gtg tgt cgg ccg gcg ggg att cag gat gaa gat ata aca ctt cag ata   1420
Val Cys Arg Pro Ala Gly Ile Gln Asp Glu Asp Ile Thr Leu Gln Ile
        410                 415                 420

act gag agt gaa gac aac gaa gaa gat gac atg gtt gat gtt atc tgg   1468
Thr Glu Ser Glu Asp Asn Glu Glu Asp Asp Met Val Asp Val Ile Trp
    425                 430                 435

cgt cag ttg ata tca agc tgc cca tgg ctt tca gaa ctt gat gaa agt   1516
Arg Gln Leu Ile Ser Ser Cys Pro Trp Leu Ser Glu Leu Asp Glu Ser
440                 445                 450                 455

gca act gaa gga gtt att aaa gtg tgg agg aaa gtt gta gat aga ata   1564
```

```
Ala Thr Glu Gly Val Ile Lys Val Trp Arg Lys Val Val Asp Arg Ile
                460                 465                 470

ttc agc ctg tac aaa ctc tct tgc agt gca tac ttt act ttc ctt aaa   1612
Phe Ser Leu Tyr Lys Leu Ser Cys Ser Ala Tyr Phe Thr Phe Leu Lys
            475                 480                 485

ctc aac gct ggt caa att cct tta gat gag gat gac cct agg ctg cat   1660
Leu Asn Ala Gly Gln Ile Pro Leu Asp Glu Asp Asp Pro Arg Leu His
        490                 495                 500

tta agt cac aga gtg gaa cag agc act gat gac atg att gtg atg gcc   1708
Leu Ser His Arg Val Glu Gln Ser Thr Asp Asp Met Ile Val Met Ala
    505                 510                 515

aca ttg cgc ctg ctg cgg ttg ctc gtg aag cac gct ggt gag ctt cgg   1756
Thr Leu Arg Leu Leu Arg Leu Leu Val Lys His Ala Gly Glu Leu Arg
520                 525                 530                 535

cag tat ctg gag cac ggc ttg gag aca aca ccc act gca cca tgg aga   1804
Gln Tyr Leu Glu His Gly Leu Glu Thr Thr Pro Thr Ala Pro Trp Arg
                540                 545                 550

gga att att ccg caa ctt ttc tca cgc tta aac cac cct gaa gtg tat   1852
Gly Ile Ile Pro Gln Leu Phe Ser Arg Leu Asn His Pro Glu Val Tyr
            555                 560                 565

gtg cgc caa agt att tgt aac ctt ctc tgc cgt gtg gct caa gat tcc   1900
Val Arg Gln Ser Ile Cys Asn Leu Leu Cys Arg Val Ala Gln Asp Ser
        570                 575                 580

cca cat ctc ata ttg tat cct gca ata gtg ggt acc ata tcg ctt agt   1948
Pro His Leu Ile Leu Tyr Pro Ala Ile Val Gly Thr Ile Ser Leu Ser
    585                 590                 595

agt gaa tcc cag gct tca gga aat aaa ttt tcc act gca att cca act   1996
Ser Glu Ser Gln Ala Ser Gly Asn Lys Phe Ser Thr Ala Ile Pro Thr
600                 605                 610                 615

tta ctt ggc aat att caa gga gaa gaa ttg ctg gtt tct gaa tgt gag   2044
Leu Leu Gly Asn Ile Gln Gly Glu Glu Leu Leu Val Ser Glu Cys Glu
                620                 625                 630

gga gga agt cct cct gca tct cag gat agc aat aag gat gaa cct aaa   2092
Gly Gly Ser Pro Pro Ala Ser Gln Asp Ser Asn Lys Asp Glu Pro Lys
            635                 640                 645

agt gga tta aat gaa gac caa gcc atg atg cag gat tgt tac agc aaa   2140
Ser Gly Leu Asn Glu Asp Gln Ala Met Met Gln Asp Cys Tyr Ser Lys
```

```
        650                 655                 660

att gta gat aag ctg tcc tct gca aac ccc acc atg gta tta cag gtt   2188
Ile Val Asp Lys Leu Ser Ser Ala Asn Pro Thr Met Val Leu Gln Val
    665                 670                 675

cag atg ctc gtg gct gaa ctg cgc agg gtc act gtg ctc tgg gat gag   2236
Gln Met Leu Val Ala Glu Leu Arg Arg Val Thr Val Leu Trp Asp Glu
680                 685                 690                 695

ctc tgg ctg gga gtt ttg ctg caa caa cac atg tat gtc ctg aga cga   2284
Leu Trp Leu Gly Val Leu Leu Gln Gln His Met Tyr Val Leu Arg Arg
                700                 705                 710

att cag cag ctt gaa gat gag gtg aag aga gtc cag aac aac aac acc   2332
Ile Gln Gln Leu Glu Asp Glu Val Lys Arg Val Gln Asn Asn Asn Thr
            715                 720                 725

tta cgc aaa gaa gag aaa att gca atc atg agg gag aag cac aca gct   2380
Leu Arg Lys Glu Glu Lys Ile Ala Ile Met Arg Glu Lys His Thr Ala
        730                 735                 740

ttg atg aag ccc atc gta ttt gct ttg gag cat gtg agg agt atc aca   2428
Leu Met Lys Pro Ile Val Phe Ala Leu Glu His Val Arg Ser Ile Thr
    745                 750                 755

gcg gct cct gca gaa aca cct cat gaa aaa tgg ttt cag gat aac tat   2476
Ala Ala Pro Ala Glu Thr Pro His Glu Lys Trp Phe Gln Asp Asn Tyr
760                 765                 770                 775

ggt gat gcc att gaa aat gcc cta gaa aaa ctg aag act cca ttg aac   2524
Gly Asp Ala Ile Glu Asn Ala Leu Glu Lys Leu Lys Thr Pro Leu Asn
                780                 785                 790

cct gca aag cct ggg agc agc tgg att cca ttt aaa gag ata atg cta   2572
Pro Ala Lys Pro Gly Ser Ser Trp Ile Pro Phe Lys Glu Ile Met Leu
            795                 800                 805

aat ttg caa cag aga gca cag aaa cgt gca agt tac atc ttg cgt ctt   2620
Asn Leu Gln Gln Arg Ala Gln Lys Arg Ala Ser Tyr Ile Leu Arg Leu
        810                 815                 820

gaa gaa atc agt cca tgg ttg gct gcc atg act aac act gaa att gct   2668
Glu Glu Ile Ser Pro Trp Leu Ala Ala Met Thr Asn Thr Glu Ile Ala
    825                 830                 835

ctt cct ggg gaa gtc tca gcc aga gac act gtc aca atc cat agt gtg   2716
Leu Pro Gly Glu Val Ser Ala Arg Asp Thr Val Thr Ile His Ser Val
840                 845                 850                 855
```

```
ggc gga acc atc aca atc tta ccg act aaa acc aag cca aag aaa ctt   2764
Gly Gly Thr Ile Thr Ile Leu Pro Thr Lys Thr Lys Pro Lys Lys Leu
                860                 865                 870

ctc ttt ctt gga tca gat ggg aag agc tat cct tat ctt ttc aaa gga   2812
Leu Phe Leu Gly Ser Asp Gly Lys Ser Tyr Pro Tyr Leu Phe Lys Gly
            875                 880                 885

ctg gag gat tta cat ctg gat gag aga ata atg cag ttc cta tct att   2860
Leu Glu Asp Leu His Leu Asp Glu Arg Ile Met Gln Phe Leu Ser Ile
        890                 895                 900

gtg aat acc atg ttt gct aca att aat cgc caa gaa aca ccc cgg ttc   2908
Val Asn Thr Met Phe Ala Thr Ile Asn Arg Gln Glu Thr Pro Arg Phe
    905                 910                 915

cat gct cga cac tat tct gta aca cca cta gga aca aga tca gga cta   2956
His Ala Arg His Tyr Ser Val Thr Pro Leu Gly Thr Arg Ser Gly Leu
920                 925                 930                 935

atc cag tgg gta gat gga gcc aca ccc tta ttt ggt ctt tac aaa cga   3004
Ile Gln Trp Val Asp Gly Ala Thr Pro Leu Phe Gly Leu Tyr Lys Arg
                940                 945                 950

tgg caa caa cgg gaa gct gcc tta caa gca caa aag gcc caa gat tcc   3052
Trp Gln Gln Arg Glu Ala Ala Leu Gln Ala Gln Lys Ala Gln Asp Ser
            955                 960                 965

tac caa act cct cag aat cct gga att gta ccc cgt cct agt gaa ctt   3100
Tyr Gln Thr Pro Gln Asn Pro Gly Ile Val Pro Arg Pro Ser Glu Leu
        970                 975                 980

tat tac agt aaa att ggc cct gct ttg aaa aca gtt ggg ctt agc ctg   3148
Tyr Tyr Ser Lys Ile Gly Pro Ala Leu Lys Thr Val Gly Leu Ser Leu
    985                 990                 995

gat gtg tcc cgt cgg gat tgg cct ctt cat gta atg aag gca gta ttg   3196
Asp Val Ser Arg Arg Asp Trp Pro Leu His Val Met Lys Ala Val Leu
1000                1005                1010                1015

gaa gag tta atg gag gcc aca ccc ccg aat ctc ctt gcc aaa gag ctc   3244
Glu Glu Leu Met Glu Ala Thr Pro Pro Asn Leu Leu Ala Lys Glu Leu
                1020                1025                1030

tgg tca tct tgc aca aca cct gat gaa tgg tgg aga gtt acg cag tct   3292
Trp Ser Ser Cys Thr Thr Pro Asp Glu Trp Trp Arg Val Thr Gln Ser
            1035                1040                1045
```

```
tat gca aga tct act gca gtc atg tct atg gtt gga tac ata att ggc   3340
Tyr Ala Arg Ser Thr Ala Val Met Ser Met Val Gly Tyr Ile Ile Gly
      1050                1055                1060

ctt gga gac aga cat ctg gat aat gtt ctt ata gat atg acg act gga   3388
Leu Gly Asp Arg His Leu Asp Asn Val Leu Ile Asp Met Thr Thr Gly
  1065                1070                1075

gaa gtt gtt cac ata gat tac aat gtt tgc ttt gaa aaa ggt aaa agc   3436
Glu Val Val His Ile Asp Tyr Asn Val Cys Phe Glu Lys Gly Lys Ser
1080                1085                1090                1095

ctt aga gtt cct gag aaa gta cct ttt cga atg aca caa aac att gaa   3484
Leu Arg Val Pro Glu Lys Val Pro Phe Arg Met Thr Gln Asn Ile Glu
                1100                1105                1110

aca gca ctg ggt gta act gga gta gaa ggt gta ttt agg ctt tca tgt   3532
Thr Ala Leu Gly Val Thr Gly Val Glu Gly Val Phe Arg Leu Ser Cys
          1115                1120                1125

gag cag gtt tta cac att atg cgg cgt ggc aga gag acc ctg ctg acg   3580
Glu Gln Val Leu His Ile Met Arg Arg Gly Arg Glu Thr Leu Leu Thr
      1130                1135                1140

ctg ctg gag gcc ttt gtg tac gac cct ctg gtg gac tgg aca gca gga   3628
Leu Leu Glu Ala Phe Val Tyr Asp Pro Leu Val Asp Trp Thr Ala Gly
  1145                1150                1155

ggc gag gct ggg ttt gct ggt gct gtc tat ggt gga ggt ggc cag cag   3676
Gly Glu Ala Gly Phe Ala Gly Ala Val Tyr Gly Gly Gly Gly Gln Gln
1160                1165                1170                1175

gcc gag agc aag cag agc aag aga gag atg gag cga gag atc acc cgc   3724
Ala Glu Ser Lys Gln Ser Lys Arg Glu Met Glu Arg Glu Ile Thr Arg
                1180                1185                1190

agc ctg ttt tct tct aga gta gct gag att aag gtg aac tgg ttt aag   3772
Ser Leu Phe Ser Ser Arg Val Ala Glu Ile Lys Val Asn Trp Phe Lys
          1195                1200                1205

aat aga gat gag atg ctg gtt gtg ctt ccc aag ttg gac ggt agc tta   3820
Asn Arg Asp Glu Met Leu Val Val Leu Pro Lys Leu Asp Gly Ser Leu
      1210                1215                1220

gat gaa tac cta agc ttg caa gag caa ctg aca gat gtg gaa aaa ctg   3868
Asp Glu Tyr Leu Ser Leu Gln Glu Gln Leu Thr Asp Val Glu Lys Leu
  1225                1230                1235

cag ggc aaa cta ctg gag gaa ata gag ttt cta gaa gga gct gaa ggg   3916
```

```
Gln Gly Lys Leu Leu Glu Glu Ile Glu Phe Leu Glu Gly Ala Glu Gly
1240                1245                1250                1255

gtg gat cat cct tct cat act ctg caa cac agg tat tct gag cac acc   3964
Val Asp His Pro Ser His Thr Leu Gln His Arg Tyr Ser Glu His Thr
                 1260                1265                1270

caa cta cag act cag caa aga gct gtt cag gaa gca atc cag gtg aag   4012
Gln Leu Gln Thr Gln Gln Arg Ala Val Gln Glu Ala Ile Gln Val Lys
            1275                1280                1285

ctg aat gaa ttt gaa caa tgg ata aca cat tat cag gct gca ttc aat   4060
Leu Asn Glu Phe Glu Gln Trp Ile Thr His Tyr Gln Ala Ala Phe Asn
        1290                1295                1300

aat tta gaa gca aca cag ctt gca agc ttg ctt caa gag ata agc aca   4108
Asn Leu Glu Ala Thr Gln Leu Ala Ser Leu Leu Gln Glu Ile Ser Thr
    1305                1310                1315

caa atg gac ctt ggt cct cca agt tac gtg cca gca aca gcc ttt ctg   4156
Gln Met Asp Leu Gly Pro Pro Ser Tyr Val Pro Ala Thr Ala Phe Leu
1320                1325                1330                1335

cag aat gct ggt cag gcc cac ttg att agc cag tgc gag cag ctg gag   4204
Gln Asn Ala Gly Gln Ala His Leu Ile Ser Gln Cys Glu Gln Leu Glu
                 1340                1345                1350

ggg gag gtt ggt gct ctc ctg cag cag agg cgc tcc gtg ctc cgt ggc   4252
Gly Glu Val Gly Ala Leu Leu Gln Gln Arg Arg Ser Val Leu Arg Gly
            1355                1360                1365

tgt ctg gag caa ctg cat cac tat gca acc gtg gcc ctg cag tat ccg   4300
Cys Leu Glu Gln Leu His His Tyr Ala Thr Val Ala Leu Gln Tyr Pro
        1370                1375                1380

aag gcc ata ttt cag aaa cat cga att gaa cag tgg aag acc tgg atg   4348
Lys Ala Ile Phe Gln Lys His Arg Ile Glu Gln Trp Lys Thr Trp Met
    1385                1390                1395

gaa gag ctc atc tgt aac acc aca gta gag cgt tgt caa gag ctc tat   4396
Glu Glu Leu Ile Cys Asn Thr Thr Val Glu Arg Cys Gln Glu Leu Tyr
1400                1405                1410                1415

agg aaa tat gaa atg caa tat gct ccc cag cca ccc cca aca gtg tgt   4444
Arg Lys Tyr Glu Met Gln Tyr Ala Pro Gln Pro Pro Pro Thr Val Cys
                 1420                1425                1430

cag ttc atc act gcc act gaa atg acc ctg cag cga tac gca gca gac   4492
Gln Phe Ile Thr Ala Thr Glu Met Thr Leu Gln Arg Tyr Ala Ala Asp
```

```
                1435                1440                1445

atc aac agc aga ctt att aga caa gtg gaa cgc ttg aaa cag gaa gct   4540
Ile Asn Ser Arg Leu Ile Arg Gln Val Glu Arg Leu Lys Gln Glu Ala
      1450                1455                1460

gtc act gtg cca gtt tgt gaa gat cag ttg aaa gaa att gaa cgt tgc   4588
Val Thr Val Pro Val Cys Glu Asp Gln Leu Lys Glu Ile Glu Arg Cys
   1465                1470                1475

att aaa gtt ttc ctt cat gag aat gga gaa gaa gga tct ttg agt cta   4636
Ile Lys Val Phe Leu His Glu Asn Gly Glu Glu Gly Ser Leu Ser Leu
1480                1485                1490                1495

gca agt gtt att att tct gcc ctt tgt acc ctt aca agg cgt aac ctg   4684
Ala Ser Val Ile Ile Ser Ala Leu Cys Thr Leu Thr Arg Arg Asn Leu
               1500                1505                1510

atg atg gaa ggt gca gcg tca agt gct gga gaa cag ctg gtt gat ctg   4732
Met Met Glu Gly Ala Ala Ser Ser Ala Gly Glu Gln Leu Val Asp Leu
          1515                1520                1525

act tct cgg gat gga gcc tgg ttc ttg gag gaa ctc tgc agt atg agc   4780
Thr Ser Arg Asp Gly Ala Trp Phe Leu Glu Glu Leu Cys Ser Met Ser
      1530                1535                1540

gga aac gtc acc tgc ttg gtt cag tta ctg aag cag tgc cac ctg gtg   4828
Gly Asn Val Thr Cys Leu Val Gln Leu Leu Lys Gln Cys His Leu Val
   1545                1550                1555

cca cag gac tta gat atc ccg aac ccc atg gaa gcg tct gag aca gtt   4876
Pro Gln Asp Leu Asp Ile Pro Asn Pro Met Glu Ala Ser Glu Thr Val
1560                1565                1570                1575

cac tta gcc aat gga gtg tat acc tca ctt cag gaa ttg aat tcg aat   4924
His Leu Ala Asn Gly Val Tyr Thr Ser Leu Gln Glu Leu Asn Ser Asn
               1580                1585                1590

ttc cgg caa atc ata ttt cca gaa gca ctt cga tgt tta atg aaa ggg   4972
Phe Arg Gln Ile Ile Phe Pro Glu Ala Leu Arg Cys Leu Met Lys Gly
          1595                1600                1605

gaa tac acg tta gaa agt atg ctg cat gaa ctg gac ggt ctt att gag   5020
Glu Tyr Thr Leu Glu Ser Met Leu His Glu Leu Asp Gly Leu Ile Glu
      1610                1615                1620

cag acc acc gat ggc gtt ccc ctg cag act cta gtg gaa tct ctt cag   5068
Gln Thr Thr Asp Gly Val Pro Leu Gln Thr Leu Val Glu Ser Leu Gln
   1625                1630                1635
```

```
gcc tac tta aga aac gca gct atg gga ctg gaa gaa gaa aca cat gct   5116
Ala Tyr Leu Arg Asn Ala Ala Met Gly Leu Glu Glu Glu Thr His Ala
1640                1645                1650                1655

cat tac atc gat gtt gcc aga cta cta cat gct cag tac ggt gaa tta   5164
His Tyr Ile Asp Val Ala Arg Leu Leu His Ala Gln Tyr Gly Glu Leu
                  1660                1665                1670

atc caa ccg aga aat ggt tca gtt gat gaa aca ccc aaa atg tca gct   5212
Ile Gln Pro Arg Asn Gly Ser Val Asp Glu Thr Pro Lys Met Ser Ala
            1675                1680                1685

ggc cag atg ctt ttg gta gca ttc gat ggc atg ttt gct caa gtt gaa   5260
Gly Gln Met Leu Leu Val Ala Phe Asp Gly Met Phe Ala Gln Val Glu
      1690                1695                1700

act gct ttc agc tta tta gtt gaa aag ttg aac aag atg gaa att ccc   5308
Thr Ala Phe Ser Leu Leu Val Glu Lys Leu Asn Lys Met Glu Ile Pro
  1705                1710                1715

ata gct tgg cga aag att gac atc ata agg gaa gcc agg agt act caa   5356
Ile Ala Trp Arg Lys Ile Asp Ile Ile Arg Glu Ala Arg Ser Thr Gln
1720                1725                1730                1735

gtt aat ttt ttt gat gat gat aat cac cgg cag gtg cta gaa gag att   5404
Val Asn Phe Phe Asp Asp Asp Asn His Arg Gln Val Leu Glu Glu Ile
                  1740                1745                1750

ttc ttt cta aaa aga cta cag act att aag gag ttc ttc agg ctc tgt   5452
Phe Phe Leu Lys Arg Leu Gln Thr Ile Lys Glu Phe Phe Arg Leu Cys
            1755                1760                1765

ggt acc ttt tct aaa aca ttg tca gga tca agt tca ctt gaa gat cag   5500
Gly Thr Phe Ser Lys Thr Leu Ser Gly Ser Ser Ser Leu Glu Asp Gln
      1770                1775                1780

aat act gtg aat ggg cct gta cag att gtc aat gtg aaa acc ctt ttt   5548
Asn Thr Val Asn Gly Pro Val Gln Ile Val Asn Val Lys Thr Leu Phe
  1785                1790                1795

aga aac tct tgt ttc agt gaa gac caa atg gcc aaa cct atc aag gca   5596
Arg Asn Ser Cys Phe Ser Glu Asp Gln Met Ala Lys Pro Ile Lys Ala
1800                1805                1810                1815

ttc aca gct gac ttt gtg agg cag ctc ttg ata ggg cta ccc aac caa   5644
Phe Thr Ala Asp Phe Val Arg Gln Leu Leu Ile Gly Leu Pro Asn Gln
                  1820                1825                1830
```

```
gcc ctc gga ctc aca ctg tgc agt ttt atc agt gct ctg ggt gta gac   5692
Ala Leu Gly Leu Thr Leu Cys Ser Phe Ile Ser Ala Leu Gly Val Asp
          1835                1840                1845

atc att gct caa gta gag gca aag gac ttt ggt gcc gaa agc aaa gtt   5740
Ile Ile Ala Gln Val Glu Ala Lys Asp Phe Gly Ala Glu Ser Lys Val
      1850                1855                1860

tct gtt gat gat ctc tgt aag aaa gcg gtg gaa cat aac atc cag ata   5788
Ser Val Asp Asp Leu Cys Lys Lys Ala Val Glu His Asn Ile Gln Ile
  1865                1870                1875

ggg aag ttc tct cag ctg gtt atg aac agg gca act gtg tta gca agt   5836
Gly Lys Phe Ser Gln Leu Val Met Asn Arg Ala Thr Val Leu Ala Ser
1880                1885                1890                1895

tct tac gac act gcc tgg aag aag cat gac ttg gtg cga agg cta gaa   5884
Ser Tyr Asp Thr Ala Trp Lys Lys His Asp Leu Val Arg Arg Leu Glu
              1900                1905                1910

acc agt att tct tct tgt aag aca agc ctg cag cgg gtt cag ctg cat   5932
Thr Ser Ile Ser Ser Cys Lys Thr Ser Leu Gln Arg Val Gln Leu His
          1915                1920                1925

att gcc atg ttt cag tgg caa cat gaa gat cta ctt atc aat aga cca   5980
Ile Ala Met Phe Gln Trp Gln His Glu Asp Leu Leu Ile Asn Arg Pro
      1930                1935                1940

caa gcc atg tca gtc aca cct ccc cca cgg tct gct atc cta acc agc   6028
Gln Ala Met Ser Val Thr Pro Pro Pro Arg Ser Ala Ile Leu Thr Ser
  1945                1950                1955

atg aaa aag aag ctg cat acc ctg agc cag att gaa act tct att gcg   6076
Met Lys Lys Lys Leu His Thr Leu Ser Gln Ile Glu Thr Ser Ile Ala
1960                1965                1970                1975

aca gtt cag gag aag cta gct gca ctt gaa tca agt att gaa cag cga   6124
Thr Val Gln Glu Lys Leu Ala Ala Leu Glu Ser Ser Ile Glu Gln Arg
              1980                1985                1990

ctc aag tgg gca ggt ggt gcc aac cct gca ttg gcc cct gta cta caa   6172
Leu Lys Trp Ala Gly Gly Ala Asn Pro Ala Leu Ala Pro Val Leu Gln
          1995                2000                2005

gat ttt gaa gca acg ata gct gaa aga aga aat ctt gtc ctt aaa gag   6220
Asp Phe Glu Ala Thr Ile Ala Glu Arg Arg Asn Leu Val Leu Lys Glu
      2010                2015                2020

agc caa aga gca agt cag gtc aca ttt ctc tgc agc aat atc att cat   6268
```

```
Ser Gln Arg Ala Ser Gln Val Thr Phe Leu Cys Ser Asn Ile Ile His
  2025                2030                2035

ttt gaa agt tta cga aca aga act gca gaa gcc tta aac ctg gat gcg  6316
Phe Glu Ser Leu Arg Thr Arg Thr Ala Glu Ala Leu Asn Leu Asp Ala
2040                2045                2050                2055

gcg tta ttt gaa cta atc aag cga tgt cag cag atg tgt tcg ttt gca  6364
Ala Leu Phe Glu Leu Ile Lys Arg Cys Gln Gln Met Cys Ser Phe Ala
                2060                2065                2070

tca cag ttt aac agt tca gtg tct gag tta gag ctt cgt tta tta cag  6412
Ser Gln Phe Asn Ser Ser Val Ser Glu Leu Glu Leu Arg Leu Leu Gln
          2075                2080                2085

aga gtg gac act ggt ctt gaa cat cct att ggc agc tct gaa tgg ctt  6460
Arg Val Asp Thr Gly Leu Glu His Pro Ile Gly Ser Ser Glu Trp Leu
       2090                2095                2100

ttg tca gca cac aaa cag ttg acc cag gat atg tct act cag agg gca  6508
Leu Ser Ala His Lys Gln Leu Thr Gln Asp Met Ser Thr Gln Arg Ala
  2105                2110                2115

att cag aca gag aaa gag cag cag ata gaa acg gtc tgt gaa aca att  6556
Ile Gln Thr Glu Lys Glu Gln Gln Ile Glu Thr Val Cys Glu Thr Ile
2120                2125                2130                2135

cag aat ctg gtt gat aat ata aag act gtg ctc act ggt cat aac cga  6604
Gln Asn Leu Val Asp Asn Ile Lys Thr Val Leu Thr Gly His Asn Arg
                2140                2145                2150

cag ctt gga gat gtc aaa cat ctc ttg aaa gct atg gct aag gat gaa  6652
Gln Leu Gly Asp Val Lys His Leu Leu Lys Ala Met Ala Lys Asp Glu
          2155                2160                2165

gaa gct gct ctg gca gat ggt gaa gat gtt ccc tat gag aac agt gtt  6700
Glu Ala Ala Leu Ala Asp Gly Glu Asp Val Pro Tyr Glu Asn Ser Val
       2170                2175                2180

agg cag ttt ttg ggt gaa tat aaa tca tgg caa gac aac att caa aca  6748
Arg Gln Phe Leu Gly Glu Tyr Lys Ser Trp Gln Asp Asn Ile Gln Thr
  2185                2190                2195

gtt cta ttt aca tta gtc cag gct atg ggt cag gtt cga agt caa gaa  6796
Val Leu Phe Thr Leu Val Gln Ala Met Gly Gln Val Arg Ser Gln Glu
2200                2205                2210                2215

cac gtt gaa atg ctc cag gaa atc act ccc acc ttg aaa gaa ctg aaa  6844
His Val Glu Met Leu Gln Glu Ile Thr Pro Thr Leu Lys Glu Leu Lys
```

```
                2220                2225                2230

aca caa agt cag agt atc tat aat aat tta gtg agt ttt gca tca ccc   6892
Thr Gln Ser Gln Ser Ile Tyr Asn Asn Leu Val Ser Phe Ala Ser Pro
          2235                2240                2245

tta gtc acc gat gca aca aat gaa tgt tcg agt cca acg tca tct gct   6940
Leu Val Thr Asp Ala Thr Asn Glu Cys Ser Ser Pro Thr Ser Ser Ala
      2250                2255                2260

act tat cag cca tcc ttc gct gca gca gtc cgg agt aac act ggc cag   6988
Thr Tyr Gln Pro Ser Phe Ala Ala Ala Val Arg Ser Asn Thr Gly Gln
  2265                2270                2275

aag act cag cct gat gtc atg tca cag aat gct aga aag ctg atc cag   7036
Lys Thr Gln Pro Asp Val Met Ser Gln Asn Ala Arg Lys Leu Ile Gln
2280                2285                2290                2295

aaa aat ctt gct aca tca gct gat act cca cca agc acc gtt cca gga   7084
Lys Asn Leu Ala Thr Ser Ala Asp Thr Pro Pro Ser Thr Val Pro Gly
              2300                2305                2310

act ggc aag agt gtt gct tgt agt cct aaa aag gca gtc aga gac cct   7132
Thr Gly Lys Ser Val Ala Cys Ser Pro Lys Lys Ala Val Arg Asp Pro
          2315                2320                2325

aaa act ggg aaa gcg gtg caa gag aga aac tcc tat gca gtg agt gtg   7180
Lys Thr Gly Lys Ala Val Gln Glu Arg Asn Ser Tyr Ala Val Ser Val
      2330                2335                2340

tgg aag aga gtg aaa gcc aag tta gag ggc cga gat gtt gat ccg aat   7228
Trp Lys Arg Val Lys Ala Lys Leu Glu Gly Arg Asp Val Asp Pro Asn
  2345                2350                2355

agg agg atg tca gtt gct gaa cag gtt gac tat gtc att aag gaa gca   7276
Arg Arg Met Ser Val Ala Glu Gln Val Asp Tyr Val Ile Lys Glu Ala
2360                2365                2370                2375

act aat cta gat aac ttg gct cag ctg tat gaa ggt tgg aca gcc tgg   7324
Thr Asn Leu Asp Asn Leu Ala Gln Leu Tyr Glu Gly Trp Thr Ala Trp
              2380                2385                2390

gtg tgaatggcaa gacagtagat gagtctggtt aagcgaggtc agacatccac        7377
Val

cagaatcaac tcagcctcag gcatccaaag ccacaccaca gtcggtggtg atgcaactgg 7437

gggcttactc tgaggaaacc taggaaatct cggtgcacta ggaagtgaat cccgcaggac 7497
```

agctgcactc agggatacgc ccaacaccat ggcctgcaac cccagggtca agggtgaagg 7557

aaagcaagct caccgcctga acacggagat tgtctttctg ccacagaaca gcagcagacg 7617

tgtcgggagg ttagctgcgg aaagaaatcg ggatgccgcg gagcacagag tgatttggaa 7677

ctccattcca cctgaccctg tgtgtacaat ccaggaaaaa aacaaacccc actcagaaac 7737

agagaaaact ggggtcgcga agaaatcaca gccaaggaag atttgatgca ttcagattct 7797

cgtgtaacac ttgttgcttg gcaacagtac tggttgggtt gaccagtaag tagaaaaagg 7857

ctaaaggcta tgcgatatga atttcagaaa tggactgaaa atggagagct atgtaacaga 7917

tacactacag tagaagaact tacttctgaa atgaagggaa aaaaaccacc ccatcgttcc 7977

ctactcctcc ccaccactta cccgttcccc ctttacctaa tctagtagat tagccatctt 8037

tcaaattcac ttttatttca gtccttatat ttcatatact tccgtctcga tgctgttaac 8097

aacttctgat aacatggaaa attcaaggat tgtttaaagg tctgatgatc acacacaaaa 8157

tgtaattccg gttatttaag tcatttctgt gattctatca tgtacagttt ccagaattgt 8217

cactgtgcat tcaaaagtaa tgaatctaac agacatttga tttaatgtac actccctttt 8277

gcttatagtg tgcatttttt ttggaggtca ttcaaatttt ccctcttctg tgatagctgt 8337

agtttctttc atagaaagta gctaatccag tgtaatcttt tacctttta aaaaccaaga 8397

tagagtatct attagagttt tacattgttg atgatagatt aacaataaag tgatgttctg 8457

gtggaggtag actgaaattt ttttaattca tgtttttcat ttgatacttt taatttacac 8517

ttagtaaatt aaaagttgtt taatttactt ggcattttag gacatgtaca tgaaacagtg 8577

aaaatgagat ccaccaacat cttttattaa gttcagttat tagtctgtga agtgctttac 8637

tttttgcaca attttaatag cttgctattc agtaatacat tatagtgaat tcatgatcaa 8697

ggtttcctta aatttagcat tgcatttcag tactgactgt gtaagctaaa ttgctgatcc 8757

aaaataaaaa cccagactag aatagggttc ttaaaatcaa gtatcaatac aaaatagaac 8817

acaattaaaa tcttaattgt tggctgggca cagtggctca cgcctgtaat cccagcactt 8877

tgggaggccg aggcgggcgg atcatgaggt taggagagcg agaccatcct ggctaacacg 8937

gtgaaacccc gtctttacta aaatacaaaa aaaattagcc gggtgtggtg gcgggcgcct 8997

gtagtcccag ctactcggga ggctgaggca ggagaatggc gtgaacccag gaggcggagc 9057

ttgcagtgag ccgagattgt gccactgcac tccagcctgg gcaacagagc tagactctgt 9117

gtcaaaaata aatgactaga t 9138

<210> 4
<211> 2392
<212> PRT
<213> Homo sapiens

<400> 4

```
Met Lys Lys Leu Leu Pro Asn Met Leu Ser Pro Asp Pro Arg Glu Leu
  1               5                  10                  15

Gln Lys Ser Ile Glu Val Gln Leu Leu Arg Ser Ser Val Cys Leu Ala
             20                  25                  30

Thr Ala Leu Asn Pro Ile Glu Gln Asp Gln Lys Trp Gln Ser Ile Thr
         35                  40                  45

Glu Asn Val Val Lys Tyr Leu Lys Gln Thr Ser Arg Ile Ala Ile Gly
     50                  55                  60

Pro Leu Arg Leu Ser Thr Leu Thr Val Ser Gln Ser Leu Pro Val Leu
 65                  70                  75                  80

Ser Thr Leu Gln Leu Tyr Cys Ser Ser Ala Leu Glu Asn Thr Val Ser
                 85                  90                  95

Asn Arg Leu Ser Thr Glu Asp Cys Leu Ile Pro Leu Phe Ser Glu Ala
            100                 105                 110

Leu Arg Ser Cys Lys Gln His Asp Val Arg Pro Trp Met Gln Ala Leu
        115                 120                 125

Arg Tyr Thr Met Tyr Gln Asn Gln Leu Leu Glu Lys Ile Lys Glu Gln
    130                 135                 140

Thr Val Pro Ile Arg Ser His Leu Met Glu Leu Gly Leu Thr Ala Ala
145                 150                 155                 160

Lys Phe Ala Arg Lys Arg Gly Asn Val Ser Leu Ala Thr Arg Leu Leu
                165                 170                 175

Ala Gln Cys Ser Glu Val Gln Leu Gly Lys Thr Thr Thr Ala Gln Asp
            180                 185                 190
```

```
Leu Val Gln His Phe Lys Lys Leu Ser Thr Gln Gly Gln Val Asp Glu
        195                 200                 205

Lys Trp Gly Pro Glu Leu Asp Ile Glu Lys Thr Lys Leu Leu Tyr Thr
    210                 215                 220

Ala Gly Gln Ser Thr His Ala Met Glu Met Leu Ser Ser Cys Ala Ile
225                 230                 235                 240

Ser Phe Cys Lys Ser Val Lys Ala Glu Tyr Ala Val Ala Lys Ser Ile
                245                 250                 255

Leu Thr Leu Ala Lys Trp Ile Gln Ala Glu Trp Lys Glu Ile Ser Gly
            260                 265                 270

Gln Leu Lys Gln Val Tyr Arg Ala Gln His Gln Gln Asn Phe Thr Gly
        275                 280                 285

Leu Ser Thr Leu Ser Lys Asn Ile Leu Thr Leu Ile Glu Leu Pro Ser
    290                 295                 300

Val Asn Thr Met Glu Glu Glu Tyr Pro Arg Ile Glu Ser Glu Ser Thr
305                 310                 315                 320

Val His Ile Gly Val Gly Glu Pro Asp Phe Ile Leu Gly Gln Leu Tyr
                325                 330                 335

His Leu Ser Ser Val Gln Ala Pro Glu Val Ala Lys Ser Trp Ala Ala
            340                 345                 350

Leu Ala Ser Trp Ala Tyr Arg Trp Gly Arg Lys Val Val Asp Asn Ala
        355                 360                 365

Ser Gln Gly Glu Gly Val Arg Leu Leu Pro Arg Glu Lys Ser Glu Val
    370                 375                 380

Gln Asn Leu Leu Pro Asp Thr Ile Thr Glu Glu Glu Lys Glu Arg Ile
385                 390                 395                 400

Tyr Gly Ile Leu Gly Gln Ala Val Cys Arg Pro Ala Gly Ile Gln Asp
                405                 410                 415

Glu Asp Ile Thr Leu Gln Ile Thr Glu Ser Glu Asp Asn Glu Glu Asp
            420                 425                 430

Asp Met Val Asp Val Ile Trp Arg Gln Leu Ile Ser Ser Cys Pro Trp
        435                 440                 445
```

Leu Ser Glu Leu Asp Glu Ser Ala Thr Glu Gly Val Ile Lys Val Trp
450 455 460

Arg Lys Val Val Asp Arg Ile Phe Ser Leu Tyr Lys Leu Ser Cys Ser
465 470 475 480

Ala Tyr Phe Thr Phe Leu Lys Leu Asn Ala Gly Gln Ile Pro Leu Asp
485 490 495

Glu Asp Asp Pro Arg Leu His Leu Ser His Arg Val Glu Gln Ser Thr
500 505 510

Asp Asp Met Ile Val Met Ala Thr Leu Arg Leu Leu Arg Leu Leu Val
515 520 525

Lys His Ala Gly Glu Leu Arg Gln Tyr Leu Glu His Gly Leu Glu Thr
530 535 540

Thr Pro Thr Ala Pro Trp Arg Gly Ile Ile Pro Gln Leu Phe Ser Arg
545 550 555 560

Leu Asn His Pro Glu Val Tyr Val Arg Gln Ser Ile Cys Asn Leu Leu
565 570 575

Cys Arg Val Ala Gln Asp Ser Pro His Leu Ile Leu Tyr Pro Ala Ile
580 585 590

Val Gly Thr Ile Ser Leu Ser Ser Glu Ser Gln Ala Ser Gly Asn Lys
595 600 605

Phe Ser Thr Ala Ile Pro Thr Leu Leu Gly Asn Ile Gln Gly Glu Glu
610 615 620

Leu Leu Val Ser Glu Cys Glu Gly Gly Ser Pro Pro Ala Ser Gln Asp
625 630 635 640

Ser Asn Lys Asp Glu Pro Lys Ser Gly Leu Asn Glu Asp Gln Ala Met
645 650 655

Met Gln Asp Cys Tyr Ser Lys Ile Val Asp Lys Leu Ser Ser Ala Asn
660 665 670

Pro Thr Met Val Leu Gln Val Gln Met Leu Val Ala Glu Leu Arg Arg
675 680 685

Val Thr Val Leu Trp Asp Glu Leu Trp Leu Gly Val Leu Leu Gln Gln
690 695 700

His Met Tyr Val Leu Arg Arg Ile Gln Gln Leu Glu Asp Glu Val Lys

705 710 715 720

Arg Val Gln Asn Asn Asn Thr Leu Arg Lys Glu Glu Lys Ile Ala Ile
725 730 735

Met Arg Glu Lys His Thr Ala Leu Met Lys Pro Ile Val Phe Ala Leu
740 745 750

Glu His Val Arg Ser Ile Thr Ala Ala Pro Ala Glu Thr Pro His Glu
755 760 765

Lys Trp Phe Gln Asp Asn Tyr Gly Asp Ala Ile Glu Asn Ala Leu Glu
770 775 780

Lys Leu Lys Thr Pro Leu Asn Pro Ala Lys Pro Gly Ser Ser Trp Ile
785 790 795 800

Pro Phe Lys Glu Ile Met Leu Asn Leu Gln Gln Arg Ala Gln Lys Arg
805 810 815

Ala Ser Tyr Ile Leu Arg Leu Glu Glu Ile Ser Pro Trp Leu Ala Ala
820 825 830

Met Thr Asn Thr Glu Ile Ala Leu Pro Gly Glu Val Ser Ala Arg Asp
835 840 845

Thr Val Thr Ile His Ser Val Gly Gly Thr Ile Thr Ile Leu Pro Thr
850 855 860

Lys Thr Lys Pro Lys Lys Leu Leu Phe Leu Gly Ser Asp Gly Lys Ser
865 870 875 880

Tyr Pro Tyr Leu Phe Lys Gly Leu Glu Asp Leu His Leu Asp Glu Arg
885 890 895

Ile Met Gln Phe Leu Ser Ile Val Asn Thr Met Phe Ala Thr Ile Asn
900 905 910

Arg Gln Glu Thr Pro Arg Phe His Ala Arg His Tyr Ser Val Thr Pro
915 920 925

Leu Gly Thr Arg Ser Gly Leu Ile Gln Trp Val Asp Gly Ala Thr Pro
930 935 940

Leu Phe Gly Leu Tyr Lys Arg Trp Gln Gln Arg Glu Ala Ala Leu Gln
945 950 955 960

Ala Gln Lys Ala Gln Asp Ser Tyr Gln Thr Pro Gln Asn Pro Gly Ile
965 970 975

Val Pro Arg Pro Ser Glu Leu Tyr Tyr Ser Lys Ile Gly Pro Ala Leu
980 985 990

Lys Thr Val Gly Leu Ser Leu Asp Val Ser Arg Arg Asp Trp Pro Leu
995 1000 1005

His Val Met Lys Ala Val Leu Glu Glu Leu Met Glu Ala Thr Pro Pro
1010 1015 1020

Asn Leu Leu Ala Lys Glu Leu Trp Ser Ser Cys Thr Thr Pro Asp Glu
025 1030 1035 1040

Trp Trp Arg Val Thr Gln Ser Tyr Ala Arg Ser Thr Ala Val Met Ser
1045 1050 1055

Met Val Gly Tyr Ile Ile Gly Leu Gly Asp Arg His Leu Asp Asn Val
1060 1065 1070

Leu Ile Asp Met Thr Thr Gly Glu Val Val His Ile Asp Tyr Asn Val
1075 1080 1085

Cys Phe Glu Lys Gly Lys Ser Leu Arg Val Pro Glu Lys Val Pro Phe
1090 1095 1100

Arg Met Thr Gln Asn Ile Glu Thr Ala Leu Gly Val Thr Gly Val Glu
105 1110 1115 1120

Gly Val Phe Arg Leu Ser Cys Glu Gln Val Leu His Ile Met Arg Arg
1125 1130 1135

Gly Arg Glu Thr Leu Leu Thr Leu Leu Glu Ala Phe Val Tyr Asp Pro
1140 1145 1150

Leu Val Asp Trp Thr Ala Gly Gly Glu Ala Gly Phe Ala Gly Ala Val
1155 1160 1165

Tyr Gly Gly Gly Gly Gln Gln Ala Glu Ser Lys Gln Ser Lys Arg Glu
1170 1175 1180

Met Glu Arg Glu Ile Thr Arg Ser Leu Phe Ser Ser Arg Val Ala Glu
185 1190 1195 1200

Ile Lys Val Asn Trp Phe Lys Asn Arg Asp Glu Met Leu Val Val Leu
1205 1210 1215

Pro Lys Leu Asp Gly Ser Leu Asp Glu Tyr Leu Ser Leu Gln Glu Gln
1220 1225 1230

Leu Thr Asp Val Glu Lys Leu Gln Gly Lys Leu Leu Glu Glu Ile Glu

1235 1240 1245

Phe Leu Glu Gly Ala Glu Gly Val Asp His Pro Ser His Thr Leu Gln
1250 1255 1260

His Arg Tyr Ser Glu His Thr Gln Leu Gln Thr Gln Gln Arg Ala Val
265 1270 1275 1280

Gln Glu Ala Ile Gln Val Lys Leu Asn Glu Phe Glu Gln Trp Ile Thr
1285 1290 1295

His Tyr Gln Ala Ala Phe Asn Asn Leu Glu Ala Thr Gln Leu Ala Ser
1300 1305 1310

Leu Leu Gln Glu Ile Ser Thr Gln Met Asp Leu Gly Pro Pro Ser Tyr
1315 1320 1325

Val Pro Ala Thr Ala Phe Leu Gln Asn Ala Gly Gln Ala His Leu Ile
1330 1335 1340

Ser Gln Cys Glu Gln Leu Glu Gly Glu Val Gly Ala Leu Leu Gln Gln
345 1350 1355 1360

Arg Arg Ser Val Leu Arg Gly Cys Leu Glu Gln Leu His His Tyr Ala
1365 1370 1375

Thr Val Ala Leu Gln Tyr Pro Lys Ala Ile Phe Gln Lys His Arg Ile
1380 1385 1390

Glu Gln Trp Lys Thr Trp Met Glu Glu Leu Ile Cys Asn Thr Thr Val
1395 1400 1405

Glu Arg Cys Gln Glu Leu Tyr Arg Lys Tyr Glu Met Gln Tyr Ala Pro
1410 1415 1420

Gln Pro Pro Pro Thr Val Cys Gln Phe Ile Thr Ala Thr Glu Met Thr
425 1430 1435 1440

Leu Gln Arg Tyr Ala Ala Asp Ile Asn Ser Arg Leu Ile Arg Gln Val
1445 1450 1455

Glu Arg Leu Lys Gln Glu Ala Val Thr Val Pro Val Cys Glu Asp Gln
1460 1465 1470

Leu Lys Glu Ile Glu Arg Cys Ile Lys Val Phe Leu His Glu Asn Gly
1475 1480 1485

Glu Glu Gly Ser Leu Ser Leu Ala Ser Val Ile Ile Ser Ala Leu Cys
1490 1495 1500

Thr Leu Thr Arg Arg Asn Leu Met Met Glu Gly Ala Ala Ser Ser Ala
505 1510 1515 1520

Gly Glu Gln Leu Val Asp Leu Thr Ser Arg Asp Gly Ala Trp Phe Leu
1525 1530 1535

Glu Glu Leu Cys Ser Met Ser Gly Asn Val Thr Cys Leu Val Gln Leu
1540 1545 1550

Leu Lys Gln Cys His Leu Val Pro Gln Asp Leu Asp Ile Pro Asn Pro
1555 1560 1565

Met Glu Ala Ser Glu Thr Val His Leu Ala Asn Gly Val Tyr Thr Ser
1570 1575 1580

Leu Gln Glu Leu Asn Ser Asn Phe Arg Gln Ile Ile Phe Pro Glu Ala
585 1590 1595 1600

Leu Arg Cys Leu Met Lys Gly Glu Tyr Thr Leu Glu Ser Met Leu His
1605 1610 1615

Glu Leu Asp Gly Leu Ile Glu Gln Thr Thr Asp Gly Val Pro Leu Gln
1620 1625 1630

Thr Leu Val Glu Ser Leu Gln Ala Tyr Leu Arg Asn Ala Ala Met Gly
1635 1640 1645

Leu Glu Glu Glu Thr His Ala His Tyr Ile Asp Val Ala Arg Leu Leu
1650 1655 1660

His Ala Gln Tyr Gly Glu Leu Ile Gln Pro Arg Asn Gly Ser Val Asp
665 1670 1675 1680

Glu Thr Pro Lys Met Ser Ala Gly Gln Met Leu Leu Val Ala Phe Asp
1685 1690 1695

Gly Met Phe Ala Gln Val Glu Thr Ala Phe Ser Leu Leu Val Glu Lys
1700 1705 1710

Leu Asn Lys Met Glu Ile Pro Ile Ala Trp Arg Lys Ile Asp Ile Ile
1715 1720 1725

Arg Glu Ala Arg Ser Thr Gln Val Asn Phe Phe Asp Asp Asp Asn His
1730 1735 1740

Arg Gln Val Leu Glu Glu Ile Phe Phe Leu Lys Arg Leu Gln Thr Ile
745 1750 1755 1760

Lys Glu Phe Phe Arg Leu Cys Gly Thr Phe Ser Lys Thr Leu Ser Gly
1765 1770 1775

Ser Ser Ser Leu Glu Asp Gln Asn Thr Val Asn Gly Pro Val Gln Ile
1780 1785 1790

Val Asn Val Lys Thr Leu Phe Arg Asn Ser Cys Phe Ser Glu Asp Gln
1795 1800 1805

Met Ala Lys Pro Ile Lys Ala Phe Thr Ala Asp Phe Val Arg Gln Leu
1810 1815 1820

Leu Ile Gly Leu Pro Asn Gln Ala Leu Gly Leu Thr Leu Cys Ser Phe
825 1830 1835 1840

Ile Ser Ala Leu Gly Val Asp Ile Ile Ala Gln Val Glu Ala Lys Asp
1845 1850 1855

Phe Gly Ala Glu Ser Lys Val Ser Val Asp Asp Leu Cys Lys Lys Ala
1860 1865 1870

Val Glu His Asn Ile Gln Ile Gly Lys Phe Ser Gln Leu Val Met Asn
1875 1880 1885

Arg Ala Thr Val Leu Ala Ser Ser Tyr Asp Thr Ala Trp Lys Lys His
1890 1895 1900

Asp Leu Val Arg Arg Leu Glu Thr Ser Ile Ser Ser Cys Lys Thr Ser
905 1910 1915 1920

Leu Gln Arg Val Gln Leu His Ile Ala Met Phe Gln Trp Gln His Glu
1925 1930 1935

Asp Leu Leu Ile Asn Arg Pro Gln Ala Met Ser Val Thr Pro Pro Pro
1940 1945 1950

Arg Ser Ala Ile Leu Thr Ser Met Lys Lys Lys Leu His Thr Leu Ser
1955 1960 1965

Gln Ile Glu Thr Ser Ile Ala Thr Val Gln Glu Lys Leu Ala Ala Leu
1970 1975 1980

Glu Ser Ser Ile Glu Gln Arg Leu Lys Trp Ala Gly Gly Ala Asn Pro
985 1990 1995 2000

Ala Leu Ala Pro Val Leu Gln Asp Phe Glu Ala Thr Ile Ala Glu Arg
2005 2010 2015

Arg Asn Leu Val Leu Lys Glu Ser Gln Arg Ala Ser Gln Val Thr Phe

2020 2025 2030

Leu Cys Ser Asn Ile Ile His Phe Glu Ser Leu Arg Thr Arg Thr Ala
2035 2040 2045

Glu Ala Leu Asn Leu Asp Ala Ala Leu Phe Glu Leu Ile Lys Arg Cys
2050 2055 2060

Gln Gln Met Cys Ser Phe Ala Ser Gln Phe Asn Ser Ser Val Ser Glu
065 2070 2075 2080

Leu Glu Leu Arg Leu Leu Gln Arg Val Asp Thr Gly Leu Glu His Pro
2085 2090 2095

Ile Gly Ser Ser Glu Trp Leu Leu Ser Ala His Lys Gln Leu Thr Gln
2100 2105 2110

Asp Met Ser Thr Gln Arg Ala Ile Gln Thr Glu Lys Glu Gln Gln Ile
2115 2120 2125

Glu Thr Val Cys Glu Thr Ile Gln Asn Leu Val Asp Asn Ile Lys Thr
2130 2135 2140

Val Leu Thr Gly His Asn Arg Gln Leu Gly Asp Val Lys His Leu Leu
145 2150 2155 2160

Lys Ala Met Ala Lys Asp Glu Glu Ala Ala Leu Ala Asp Gly Glu Asp
2165 2170 2175

Val Pro Tyr Glu Asn Ser Val Arg Gln Phe Leu Gly Glu Tyr Lys Ser
2180 2185 2190

Trp Gln Asp Asn Ile Gln Thr Val Leu Phe Thr Leu Val Gln Ala Met
2195 2200 2205

Gly Gln Val Arg Ser Gln Glu His Val Glu Met Leu Gln Glu Ile Thr
2210 2215 2220

Pro Thr Leu Lys Glu Leu Lys Thr Gln Ser Gln Ser Ile Tyr Asn Asn
225 2230 2235 2240

Leu Val Ser Phe Ala Ser Pro Leu Val Thr Asp Ala Thr Asn Glu Cys
2245 2250 2255

Ser Ser Pro Thr Ser Ser Ala Thr Tyr Gln Pro Ser Phe Ala Ala Ala
2260 2265 2270

Val Arg Ser Asn Thr Gly Gln Lys Thr Gln Pro Asp Val Met Ser Gln
2275 2280 2285

Asn Ala Arg Lys Leu Ile Gln Lys Asn Leu Ala Thr Ser Ala Asp Thr
2290 2295 2300

Pro Pro Ser Thr Val Pro Gly Thr Gly Lys Ser Val Ala Cys Ser Pro
305 2310 2315 2320

Lys Lys Ala Val Arg Asp Pro Lys Thr Gly Lys Ala Val Gln Glu Arg
2325 2330 2335

Asn Ser Tyr Ala Val Ser Val Trp Lys Arg Val Lys Ala Lys Leu Glu
2340 2345 2350

Gly Arg Asp Val Asp Pro Asn Arg Arg Met Ser Val Ala Glu Gln Val
2355 2360 2365

Asp Tyr Val Ile Lys Glu Ala Thr Asn Leu Asp Asn Leu Ala Gln Leu
2370 2375 2380

Tyr Glu Gly Trp Thr Ala Trp Val
385 2390

<210> 5
<211> 2392
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: kinase inactive BLIP protein

<400> 5
Met Lys Lys Leu Leu Pro Asn Met Leu Ser Pro Asp Pro Arg Glu Leu
1 5 10 15

Gln Lys Ser Ile Glu Val Gln Leu Leu Arg Ser Ser Val Cys Leu Ala
20 25 30

Thr Ala Leu Asn Pro Ile Glu Gln Asp Gln Lys Trp Gln Ser Ile Thr
35 40 45

Glu Asn Val Val Lys Tyr Leu Lys Gln Thr Ser Arg Ile Ala Ile Gly
50 55 60

Pro Leu Arg Leu Ser Thr Leu Thr Val Ser Gln Ser Leu Pro Val Leu
65 70 75 80

Ser Thr Leu Gln Leu Tyr Cys Ser Ser Ala Leu Glu Asn Thr Val Ser

```
                  85                  90                  95

Asn Arg Leu Ser Thr Glu Asp Cys Leu Ile Pro Leu Phe Ser Glu Ala
            100                 105                 110

Leu Arg Ser Cys Lys Gln His Asp Val Arg Pro Trp Met Gln Ala Leu
        115                 120                 125

Arg Tyr Thr Met Tyr Gln Asn Gln Leu Leu Glu Lys Ile Lys Glu Gln
    130                 135                 140

Thr Val Pro Ile Arg Ser His Leu Met Glu Leu Gly Leu Thr Ala Ala
145                 150                 155                 160

Lys Phe Ala Arg Lys Arg Gly Asn Val Ser Leu Ala Thr Arg Leu Leu
                165                 170                 175

Ala Gln Cys Ser Glu Val Gln Leu Gly Lys Thr Thr Thr Ala Gln Asp
            180                 185                 190

Leu Val Gln His Phe Lys Lys Leu Ser Thr Gln Gly Gln Val Asp Glu
        195                 200                 205

Lys Trp Gly Pro Glu Leu Asp Ile Glu Lys Thr Lys Leu Leu Tyr Thr
    210                 215                 220

Ala Gly Gln Ser Thr His Ala Met Glu Met Leu Ser Ser Cys Ala Ile
225                 230                 235                 240

Ser Phe Cys Lys Ser Val Lys Ala Glu Tyr Ala Val Ala Lys Ser Ile
                245                 250                 255

Leu Thr Leu Ala Lys Trp Ile Gln Ala Glu Trp Lys Glu Ile Ser Gly
            260                 265                 270

Gln Leu Lys Gln Val Tyr Arg Ala Gln His Gln Gln Asn Phe Thr Gly
        275                 280                 285

Leu Ser Thr Leu Ser Lys Asn Ile Leu Thr Leu Ile Glu Leu Pro Ser
    290                 295                 300

Val Asn Thr Met Glu Glu Glu Tyr Pro Arg Ile Glu Ser Glu Ser Thr
305                 310                 315                 320

Val His Ile Gly Val Gly Glu Pro Asp Phe Ile Leu Gly Gln Leu Tyr
                325                 330                 335

His Leu Ser Ser Val Gln Ala Pro Glu Val Ala Lys Ser Trp Ala Ala
            340                 345                 350
```

```
Leu Ala Ser Trp Ala Tyr Arg Trp Gly Arg Lys Val Val Asp Asn Ala
        355                 360                 365

Ser Gln Gly Glu Gly Val Arg Leu Leu Pro Arg Glu Lys Ser Glu Val
    370                 375                 380

Gln Asn Leu Leu Pro Asp Thr Ile Thr Glu Glu Glu Lys Glu Arg Ile
385                 390                 395                 400

Tyr Gly Ile Leu Gly Gln Ala Val Cys Arg Pro Ala Gly Ile Gln Asp
                405                 410                 415

Glu Asp Ile Thr Leu Gln Ile Thr Glu Ser Glu Asp Asn Glu Glu Asp
            420                 425                 430

Asp Met Val Asp Val Ile Trp Arg Gln Leu Ile Ser Ser Cys Pro Trp
        435                 440                 445

Leu Ser Glu Leu Asp Glu Ser Ala Thr Glu Gly Val Ile Lys Val Trp
    450                 455                 460

Arg Lys Val Val Asp Arg Ile Phe Ser Leu Tyr Lys Leu Ser Cys Ser
465                 470                 475                 480

Ala Tyr Phe Thr Phe Leu Lys Leu Asn Ala Gly Gln Ile Pro Leu Asp
                485                 490                 495

Glu Asp Asp Pro Arg Leu His Leu Ser His Arg Val Glu Gln Ser Thr
            500                 505                 510

Asp Asp Met Ile Val Met Ala Thr Leu Arg Leu Leu Arg Leu Leu Val
        515                 520                 525

Lys His Ala Gly Glu Leu Arg Gln Tyr Leu Glu His Gly Leu Glu Thr
    530                 535                 540

Thr Pro Thr Ala Pro Trp Arg Gly Ile Ile Pro Gln Leu Phe Ser Arg
545                 550                 555                 560

Leu Asn His Pro Glu Val Tyr Val Arg Gln Ser Ile Cys Asn Leu Leu
                565                 570                 575

Cys Arg Val Ala Gln Asp Ser Pro His Leu Ile Leu Tyr Pro Ala Ile
            580                 585                 590

Val Gly Thr Ile Ser Leu Ser Ser Glu Ser Gln Ala Ser Gly Asn Lys
        595                 600                 605
Phe Ser Thr Ala Ile Pro Thr Leu Leu Gly Asn Ile Gln Gly Glu Glu
```

610 615 620

Leu Leu Val Ser Glu Cys Glu Gly Gly Ser Pro Pro Ala Ser Gln Asp
625 630 635 640

Ser Asn Lys Asp Glu Pro Lys Ser Gly Leu Asn Glu Asp Gln Ala Met
645 650 655

Met Gln Asp Cys Tyr Ser Lys Ile Val Asp Lys Leu Ser Ser Ala Asn
660 665 670

Pro Thr Met Val Leu Gln Val Gln Met Leu Val Ala Glu Leu Arg Arg
675 680 685

Val Thr Val Leu Trp Asp Glu Leu Trp Leu Gly Val Leu Leu Gln Gln
690 695 700

His Met Tyr Val Leu Arg Arg Ile Gln Gln Leu Glu Asp Glu Val Lys
705 710 715 720

Arg Val Gln Asn Asn Asn Thr Leu Arg Lys Glu Glu Lys Ile Ala Ile
725 730 735

Met Arg Glu Lys His Thr Ala Leu Met Lys Pro Ile Val Phe Ala Leu
740 745 750

Glu His Val Arg Ser Ile Thr Ala Ala Pro Ala Glu Thr Pro His Glu
755 760 765

Lys Trp Phe Gln Asp Asn Tyr Gly Asp Ala Ile Glu Asn Ala Leu Glu
770 775 780

Lys Leu Lys Thr Pro Leu Asn Pro Ala Lys Pro Gly Ser Ser Trp Ile
785 790 795 800

Pro Phe Lys Glu Ile Met Leu Asn Leu Gln Gln Arg Ala Gln Lys Arg
805 810 815

Ala Ser Tyr Ile Leu Arg Leu Glu Glu Ile Ser Pro Trp Leu Ala Ala
820 825 830

Met Thr Asn Thr Glu Ile Ala Leu Pro Gly Glu Val Ser Ala Arg Asp
835 840 845

Thr Val Thr Ile His Ser Val Gly Gly Thr Ile Thr Ile Leu Pro Thr
850 855 860

Lys Thr Lys Pro Lys Lys Leu Leu Phe Leu Gly Ser Asp Gly Lys Ser
865 870 875 880

Tyr Pro Tyr Leu Phe Lys Gly Leu Glu Asp Leu His Leu Asp Glu Arg
885 890 895

Ile Met Gln Phe Leu Ser Ile Val Asn Thr Met Phe Ala Thr Ile Asn
900 905 910

Arg Gln Glu Thr Pro Arg Phe His Ala Arg His Tyr Ser Val Thr Pro
915 920 925

Leu Gly Thr Arg Ser Gly Leu Ile Gln Trp Val Asp Gly Ala Thr Pro
930 935 940

Leu Phe Gly Leu Tyr Lys Arg Trp Gln Gln Arg Glu Ala Ala Leu Gln
945 950 955 960

Ala Gln Lys Ala Gln Asp Ser Tyr Gln Thr Pro Gln Asn Pro Gly Ile
965 970 975

Val Pro Arg Pro Ser Glu Leu Tyr Tyr Ser Lys Ile Gly Pro Ala Leu
980 985 990

Lys Thr Val Gly Leu Ser Leu Asp Val Ser Arg Arg Asp Trp Pro Leu
995 1000 1005

His Val Met Lys Ala Val Leu Glu Glu Leu Met Glu Ala Thr Pro Pro
1010 1015 1020

Asn Leu Leu Ala Lys Glu Leu Trp Ser Ser Cys Thr Thr Pro Asp Glu
1025 1030 1035 1040

Trp Trp Arg Val Thr Gln Ser Tyr Ala Arg Ser Thr Ala Val Met Ser
1045 1050 1055

Met Val Gly Tyr Ile Ile Gly Leu Gly Ala Arg His Leu Asp Lys Val
1060 1065 1070

Leu Ile Asp Met Thr Thr Gly Glu Val Val His Ile Asp Tyr Asn Val
1075 1080 1085

Cys Phe Glu Lys Gly Lys Ser Leu Arg Val Pro Glu Lys Val Pro Phe
1090 1095 1100

Arg Met Thr Gln Asn Ile Glu Thr Ala Leu Gly Val Thr Gly Val Glu
1105 1110 1115 1120

Gly Val Phe Arg Leu Ser Cys Glu Gln Val Leu His Ile Met Arg Arg
1125 1130 1135

Gly Arg Glu Thr Leu Leu Thr Leu Leu Glu Ala Phe Val Tyr Asp Pro
1140 1145 1150

Leu Val Asp Trp Thr Ala Gly Gly Glu Ala Gly Phe Ala Gly Ala Val
1155 1160 1165

Tyr Gly Gly Gly Gly Gln Gln Ala Glu Ser Lys Gln Ser Lys Arg Glu
1170 1175 1180

Met Glu Arg Glu Ile Thr Arg Ser Leu Phe Ser Ser Arg Val Ala Glu
1185 1190 1195 1200

Ile Lys Val Asn Trp Phe Lys Asn Arg Asp Glu Met Leu Val Val Leu
1205 1210 1215

Pro Lys Leu Asp Gly Ser Leu Asp Glu Tyr Leu Ser Leu Gln Glu Gln
1220 1225 1230

Leu Thr Asp Val Glu Lys Leu Gln Gly Lys Leu Leu Glu Glu Ile Glu
1235 1240 1245

Phe Leu Glu Gly Ala Glu Gly Val Asp His Pro Ser His Thr Leu Gln
1250 1255 1260

His Arg Tyr Ser Glu His Thr Gln Leu Gln Thr Gln Gln Arg Ala Val
1265 1270 1275 1280

Gln Glu Ala Ile Gln Val Lys Leu Asn Glu Phe Glu Gln Trp Ile Thr
1285 1290 1295

His Tyr Gln Ala Ala Phe Asn Asn Leu Glu Ala Thr Gln Leu Ala Ser
1300 1305 1310

Leu Leu Gln Glu Ile Ser Thr Gln Met Asp Leu Gly Pro Pro Ser Tyr
1315 1320 1325

Val Pro Ala Thr Ala Phe Leu Gln Asn Ala Gly Gln Ala His Leu Ile
1330 1335 1340

Ser Gln Cys Glu Gln Leu Glu Gly Glu Val Gly Ala Leu Leu Gln Gln
1345 1350 1355 1360

Arg Arg Ser Val Leu Arg Gly Cys Leu Glu Gln Leu His His Tyr Ala
1365 1370 1375

Thr Val Ala Leu Gln Tyr Pro Lys Ala Ile Phe Gln Lys His Arg Ile
1380 1385 1390

Glu Gln Trp Lys Thr Trp Met Glu Glu Leu Ile Cys Asn Thr Thr Val

1395 1400 1405

Glu Arg Cys Gln Glu Leu Tyr Arg Lys Tyr Glu Met Gln Tyr Ala Pro
1410 1415 1420

Gln Pro Pro Pro Thr Val Cys Gln Phe Ile Thr Ala Thr Glu Met Thr
1425 1430 1435 1440

Leu Gln Arg Tyr Ala Ala Asp Ile Asn Ser Arg Leu Ile Arg Gln Val
1445 1450 1455

Glu Arg Leu Lys Gln Glu Ala Val Thr Val Pro Val Cys Glu Asp Gln
1460 1465 1470

Leu Lys Glu Ile Glu Arg Cys Ile Lys Val Phe Leu His Glu Asn Gly
1475 1480 1485

Glu Glu Gly Ser Leu Ser Leu Ala Ser Val Ile Ile Ser Ala Leu Cys
1490 1495 1500

Thr Leu Thr Arg Arg Asn Leu Met Met Glu Gly Ala Ala Ser Ser Ala
1505 1510 1515 1520

Gly Glu Gln Leu Val Asp Leu Thr Ser Arg Asp Gly Ala Trp Phe Leu
1525 1530 1535

Glu Glu Leu Cys Ser Met Ser Gly Asn Val Thr Cys Leu Val Gln Leu
1540 1545 1550

Leu Lys Gln Cys His Leu Val Pro Gln Asp Leu Asp Ile Pro Asn Pro
1555 1560 1565

Met Glu Ala Ser Glu Thr Val His Leu Ala Asn Gly Val Tyr Thr Ser
1570 1575 1580

Leu Gln Glu Leu Asn Ser Asn Phe Arg Gln Ile Ile Phe Pro Glu Ala
1585 1590 1595 1600

Leu Arg Cys Leu Met Lys Gly Glu Tyr Thr Leu Glu Ser Met Leu His
1605 1610 1615

Glu Leu Asp Gly Leu Ile Glu Gln Thr Thr Asp Gly Val Pro Leu Gln
1620 1625 1630

Thr Leu Val Glu Ser Leu Gln Ala Tyr Leu Arg Asn Ala Ala Met Gly
1635 1640 1645

Leu Glu Glu Glu Thr His Ala His Tyr Ile Asp Val Ala Arg Leu Leu
1650 1655 1660

```
His Ala Gln Tyr Gly Glu Leu Ile Gln Pro Arg Asn Gly Ser Val Asp
1665                1670                1675                1680

Glu Thr Pro Lys Met Ser Ala Gly Gln Met Leu Leu Val Ala Phe Asp
                1685                1690                1695

Gly Met Phe Ala Gln Val Glu Thr Ala Phe Ser Leu Leu Val Glu Lys
            1700                1705                1710

Leu Asn Lys Met Glu Ile Pro Ile Ala Trp Arg Lys Ile Asp Ile Ile
        1715                1720                1725

Arg Glu Ala Arg Ser Thr Gln Val Asn Phe Phe Asp Asp Asp Asn His
    1730                1735                1740

Arg Gln Val Leu Glu Glu Ile Phe Phe Leu Lys Arg Leu Gln Thr Ile
1745                1750                1755                1760

Lys Glu Phe Phe Arg Leu Cys Gly Thr Phe Ser Lys Thr Leu Ser Gly
                1765                1770                1775

Ser Ser Ser Leu Glu Asp Gln Asn Thr Val Asn Gly Pro Val Gln Ile
            1780                1785                1790

Val Asn Val Lys Thr Leu Phe Arg Asn Ser Cys Phe Ser Glu Asp Gln
        1795                1800                1805

Met Ala Lys Pro Ile Lys Ala Phe Thr Ala Asp Phe Val Arg Gln Leu
    1810                1815                1820

Leu Ile Gly Leu Pro Asn Gln Ala Leu Gly Leu Thr Leu Cys Ser Phe
1825                1830                1835                1840

Ile Ser Ala Leu Gly Val Asp Ile Ile Ala Gln Val Glu Ala Lys Asp
                1845                1850                1855

Phe Gly Ala Glu Ser Lys Val Ser Val Asp Asp Leu Cys Lys Lys Ala
            1860                1865                1870

Val Glu His Asn Ile Gln Ile Gly Lys Phe Ser Gln Leu Val Met Asn
        1875                1880                1885

Arg Ala Thr Val Leu Ala Ser Ser Tyr Asp Thr Ala Trp Lys Lys His
    1890                1895                1900

Asp Leu Val Arg Arg Leu Glu Thr Ser Ile Ser Ser Cys Lys Thr Ser
1905                1910                1915                1920
```

Leu Gln Arg Val Gln Leu His Ile Ala Met Phe Gln Trp Gln His Glu
1925 1930 1935

Asp Leu Leu Ile Asn Arg Pro Gln Ala Met Ser Val Thr Pro Pro Pro
1940 1945 1950

Arg Ser Ala Ile Leu Thr Ser Met Lys Lys Lys Leu His Thr Leu Ser
1955 1960 1965

Gln Ile Glu Thr Ser Ile Ala Thr Val Gln Glu Lys Leu Ala Ala Leu
1970 1975 1980

Glu Ser Ser Ile Glu Gln Arg Leu Lys Trp Ala Gly Gly Ala Asn Pro
1985 1990 1995 2000

Ala Leu Ala Pro Val Leu Gln Asp Phe Glu Ala Thr Ile Ala Glu Arg
2005 2010 2015

Arg Asn Leu Val Leu Lys Glu Ser Gln Arg Ala Ser Gln Val Thr Phe
2020 2025 2030

Leu Cys Ser Asn Ile Ile His Phe Glu Ser Leu Arg Thr Arg Thr Ala
2035 2040 2045

Glu Ala Leu Asn Leu Asp Ala Ala Leu Phe Glu Leu Ile Lys Arg Cys
2050 2055 2060

Gln Gln Met Cys Ser Phe Ala Ser Gln Phe Asn Ser Ser Val Ser Glu
2065 2070 2075 2080

Leu Glu Leu Arg Leu Leu Gln Arg Val Asp Thr Gly Leu Glu His Pro
2085 2090 2095

Ile Gly Ser Ser Glu Trp Leu Leu Ser Ala His Lys Gln Leu Thr Gln
2100 2105 2110

Asp Met Ser Thr Gln Arg Ala Ile Gln Thr Glu Lys Glu Gln Gln Ile
2115 2120 2125

Glu Thr Val Cys Glu Thr Ile Gln Asn Leu Val Asp Asn Ile Lys Thr
2130 2135 2140

Val Leu Thr Gly His Asn Arg Gln Leu Gly Asp Val Lys His Leu Leu
2145 2150 2155 2160

Lys Ala Met Ala Lys Asp Glu Glu Ala Ala Leu Ala Asp Gly Glu Asp
2165 2170 2175

Val Pro Tyr Glu Asn Ser Val Arg Gln Phe Leu Gly Glu Tyr Lys Ser
2180 2185 2190

```
Trp Gln Asp Asn Ile Gln Thr Val Leu Phe Thr Leu Val Gln Ala Met
      2195                2200                2205

Gly Gln Val Arg Ser Gln Glu His Val Glu Met Leu Gln Glu Ile Thr
   2210                2215                2220

Pro Thr Leu Lys Glu Leu Lys Thr Gln Ser Gln Ser Ile Tyr Asn Asn
2225                2230                2235                2240

Leu Val Ser Phe Ala Ser Pro Leu Val Thr Asp Ala Thr Asn Glu Cys
                2245                2250                2255

Ser Ser Pro Thr Ser Ser Ala Thr Tyr Gln Pro Ser Phe Ala Ala Ala
           2260                2265                2270

Val Arg Ser Asn Thr Gly Gln Lys Thr Gln Pro Asp Val Met Ser Gln
      2275                2280                2285

Asn Ala Arg Lys Leu Ile Gln Lys Asn Leu Ala Thr Ser Ala Asp Thr
   2290                2295                2300

Pro Pro Ser Thr Val Pro Gly Thr Gly Lys Ser Val Ala Cys Ser Pro
2305                2310                2315                2320

Lys Lys Ala Val Arg Asp Pro Lys Thr Gly Lys Ala Val Gln Glu Arg
                2325                2330                2335

Asn Ser Tyr Ala Val Ser Val Trp Lys Arg Val Lys Ala Lys Leu Glu
           2340                2345                2350

Gly Arg Asp Val Asp Pro Asn Arg Arg Met Ser Val Ala Glu Gln Val
      2355                2360                2365

Asp Tyr Val Ile Lys Glu Ala Thr Asn Leu Asp Asn Leu Ala Gln Leu
   2370                2375                2380

Tyr Glu Gly Trp Thr Ala Trp Val
2385                2390
```

<210> 6
<211> 779
<212> PRT
<213> Homo sapiens

<400> 6

```
Met Leu His Glu Leu Asp Gly Leu Ile Glu Gln Thr Thr Asp Gly Val
  1               5                  10                  15
```

Claims

1. An isolated human BLIP protein or a variant thereof.

2. An isolated protein having a sequence selected from:

(a) SEQ ID NO:4; and
(b) sequences that have at least about 70% sequence similarity to SEQ ID NO:4, and wherein the protein is a BLIP protein.

3. A kinase inactive mutant of a BLIP protein according to claim 2.

4. A kinase inactive mutant of a BLIP protein having SEQ ID NO:5.

5. An isolated protein having a sequence selected from:

(a) SEQ ID NO:6; and
(b) sequences that have at least about 90% sequence similarity to SEQ ID NO:6, and wherein the protein is a LIP protein.

6. An isolated nucleic acid molecule having a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a protein of SEQ ID NO:4;
(b) a nucleotide sequence that hybridizes to the sequence of (a) above under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1 % SDS at 60 degrees C°, and which encodes a BLIP protein;
(c) a nucleotide sequence complementary to a sequence of (a) - (b), above.

7. An isolated nucleotide molecule according to claim 6 which is a cDNA sequence.

8. An isolated nucleic acid which encodes a BLIP protein having the amino acid sequence given herein as SEQ ID NO:4.

9. An isolated nucleic acid molecule having a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a protein of SEQ ID NO:6;
(b) a nucleotide sequence that hybridizes to the sequence of (a) above under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1% SDS at 60 degrees C°, and which encodes a LIP protein with at least about 90% amino acid sequence similarity to SEQ ID NO:6;
(c) a nucleotide sequence complementary to a sequence of (a) - (b), above.

10. An isolated nucleic acid which encodes a LIP protein having the amino acid sequence given herein as SEQ ID NO:6.

11. A nucleic acid construct having a promoter and a heterologous nucleic acid operably linked to said promoter, wherein said heterologous nucleic acid is a nucleic acid according to claim 6-10.

12. A cell containing a nucleic acid construct according to claim 11.

13. A cell containing a nucleic acid construct according to claim 11 and capable of expressing the encoded protein.

14. An expression vector comprising a nucleotide sequence according to any one of claims 6 to 10, which is capable of expressing the encoded protein.

15. A cell line according to claim 13 which is a modified HEK293 or HeLa cell line.

16. A method of producing a BLIP protein comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence according to claim 6, under conditions suitable for obtaining expression of the nucleotide sequence.

17. An antibody that specifically binds to a protein encoded by a nucleic acid molecule of claim 6.

18. An antibody according to claim 17 that is a monoclonal antibody.

19. A method of screening compounds for the ability to modulate BLIP-dependent NFκB activation, comprising ad-

ministering a test compound to a test cell and detecting any increase or decrease in BLIP-dependent NFκB activation in the test cell, compared to a control cell that was not administered the test compound.

20. A method according to claim 19 where said detection of BLIP-dependent NFκB activation utilizes a κB-dependent reporter gene.

21. A compound identified by the method according to claim 19.

22. A method of inhibiting NF-κB activation in a cell comprising administering to the cell an inhibitor of BLIP-related NF-κB activation, in an amount effective to inhibit NF-κB activation therein compared to the NF-κB activation that would occur in the absence of said inhibitor.

23. A method according to claim 22 wherein said inhibitor is a BLIP kinase-dead mutant or a LIP protein.

24. A method of treatment or prophylaxis of a disorder that is responsive to inhibition of BLIP-dependent NF-κB activation in a mammalian subject, comprising administering to said subject an effective amount of a compound that inhibits BLIP-dependent NF-κB activation.

25. A method according to claim 24 where said disorder is selected from neoplastic, inflammatory and immune disorders.

26. The use of a compound that modulates of BLIP-dependent NF-κB activation in a method of formulating a medicament for treatment or prophylaxis of a disorder that is responsive to modulation of BLIP-dependent NF-κB activation.

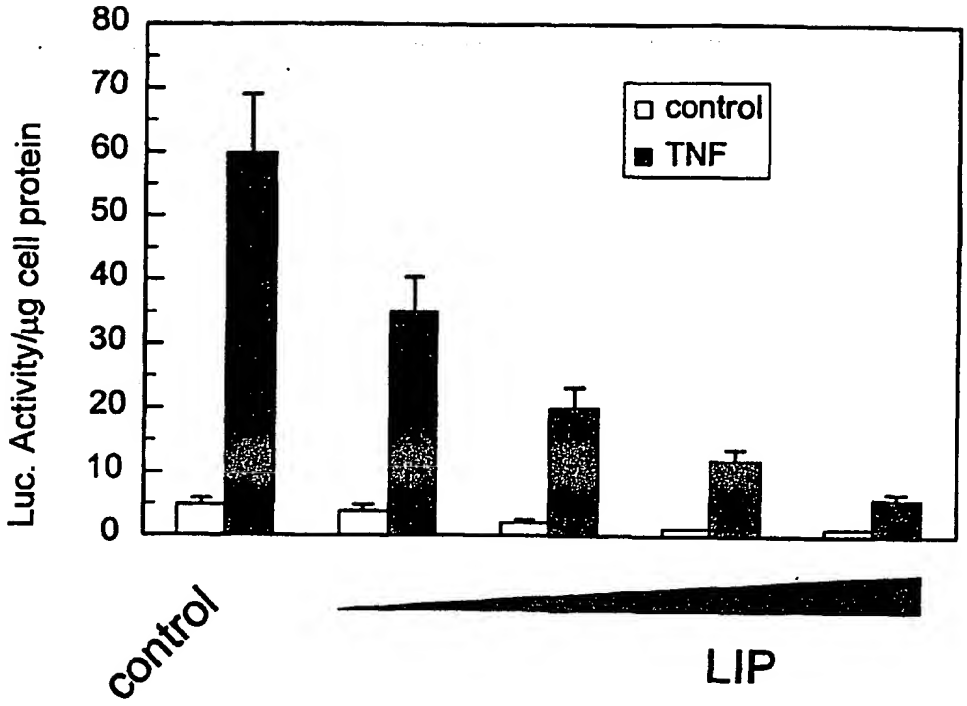
80
70
60
50
40
30
20
10
0
Luc. Activity/µg cell protein
control
TNF
control
LIP

FIG. 1

```
MKKLLPNMLS PDPRELQKSI EVQLLRSSVC LATALNPIEQ DQKWQSITEN VVKYLKQTSR    60
IAIGPLRLST LTVSQSLPVL STLQLYCSSA LENTVSNRLS TEDCLIPLFS EALRSCKQHD   120
VRPWMQALRY TMYQNQLLEK IKEQTVPIRS HLMELGLTAA KFARKRGNVS LATRLLAQCS   180
EVQLGKTTTA QDLVQHFKKL STQGQVDEKW GPELDIEKTK LLYTAGQSTH AMEMLSSCAI   240
SFCKSVKAEY AVAKSILTLA KWIQAEWKEI SGQLKQVYRA QHQQNFTGLS TLSKNILTLI   300
ELPSVNTMEE EYPRIESEST VHIGVGEPDF ILGQLYHLSS VQAPEVAKSW AALASWAYRW   360
GRKVVDNASQ GEGVRLLPRE KSEVQNLLPD TITEEEKERI YGILGQAVCR PAGIQDEDIT   420
LQITESEDNE EDDMVDVIWR QLISSCPWLS ELDESATEGV IKVWRKVVDR IFSLYKLSCS   480
AYFTFLKLNA GQIPLDEDDP RLHLSHRVEQ STDDMIVMAT LRLLRLLVKH AGELRQYLEH   540
GLETTPTAPW RGIIPQLFSR LNHPEVYVRQ SICNLLCRVA QDSPHLILYP AIVGTISLSS   600
ESQASGNKFS TAIPTLLGNI QGEELLVSEC EGGSPPASQD SNKDEPKSGL NEDQAMMQDC   660
YSKIVDKLSS ANPTMVLQVQ MLVAELRRVT VLWDELWLGV LLQQHMYVLR RIQQLEDEVK   720
RVQNNNTLRK EEKIAIMREK HTALMKPIVF ALEHVRSITA APAETPHEKW FQDNYGDAIE   780
NALEKLKTPL NPAKPGSSWI PFKEIMLNLQ QRAQKRASYI LRLEEISPWL AAMTNTEIAL   840
PGEVSARDTV TIHSVGGTIT ILPTKTKPKK LLFLGSDGKS YPYLFKGLED LHLDERIMQF   900
LSIVNTMFAT INRQETPRFH ARHYSVTPLG TRSGLIQWVD GATPLFGLYK RWQQREAALQ   960
AQKAQDSYQT PQNPGIVPRP SELYYSKIGP ALKTVGLSLD VSRRDWPLHV MKAVLEELME  1020
ATPPNLLAKE LWSSCTTPDE WWRVTQSYAR STAVMSMVGY IIGLGDRHLD NVLIDMTTGE  1080
VVHIDYNVCF EKGKSLRVPE KVPFRMTQNI ETALGVTGVE GVFRLSCEQV LHIMRRGRET  1140
LLTLLEAFVY DPLVDWTAGG EAGFAGAVYG GGGQQAESKQ SKREMEREIT RSLFSSRVAE  1200
IKVNWFKNRD EMLVVLPKLD GSLDEYLSLQ EQLTDVEKLQ GKLLEEIEFL EGAEGVDHPS  1260
HTLQHRYSEH TQLQTQQRAV QEAIQVKLNE FEQWITHYQA AFNNLEATQL ASLLQEISTQ  1320
MDLGPPSYVP ATAFLQNAGQ AHLISQCEQL EGEVGALLQQ RRSVLRGCLE QLHHYATVAL  1380
QYPKAIFQKH RIEQWKTWME ELICNTTVER CQELYRKYEM QYAPQPPPTV CQFITATEMT  1440
LQRYAADINS RLIRQVERLK QEAVTVPVCE DQLKEIERCI KVFLHENGEE GSLSLASVII  1500
SALCTLTRRN LMMEGAASSA GEQLVDLTSR DGAWFLEELC SMSGNVTCLV QLLKQCHLVP  1560
QDLDIPNPME ASETVHLANG VYTSLQELNS NFRQIIFPEA LRCLMKGEYT LESMLHELDG  1620
LIEQTTDGVP LQTLVESLQA YLRNAAMGLE EETHAHYIDV ARLLHAQYGE LIQPRNGSVD  1680
ETPKMSAGQM LLVAFDGMFA QVETAFSLLV EKLNKMEIPI AWRKIDIIRE ARSTQVNFFD  1740
DDNHRQVLEE IFFLKRLQTI KEFFRLCGTF SKTLSGSSSL EDQNTVNGPV QIVNVKTLFR  1800
NSCFSEDQMA KPIKAFTADF VRQLLIGLPN QALGLTLCSF ISALGVDIIA QVEAKDFGAE  1860
SKVSVDDLCK KAVEHNIQIG KFSQLVMNRA TVLASSYDTA WKKHDLVRRL ETSISSCKTS  1920
LQRVQLHIAM FQWQHEDLLI NRPQAMSVTP PPRSAILTSM KKKLHTLSQI ETSIATVQEK  1980
LAALESSIEQ RLKWAGGANP ALAPVLQDFE ATIAERRNLV LKESQRASQV TFLCSNIIHF  2040
ESLRTRTAEA LNLDAALFEL IKRCQQMCSF ASQFNSSVSE LELRLLQRVD TGLEHPIGSS  2100
EWLLSAHKQL TQDMSTQRAI QTEKEQQIET VCETIQNLVD NIKTVLTGHN RQLGDVKHLL  2160
KAMAKDEEAA LADGEDVPYE NSVRQFLGEY KSWQDNIQTV LFTLVQAMGQ VRSQEHVEML  2220
QEITPTLKEL KTQSQSIYNN LVSFASPLVT DATNECSSPT SSATYQPSFA AAVRSNTGQK  2280
TQPDVMSQNA RKLIQKNLAT SADTPPSTVP GTGKSVACSP KKAVRDPKTG KAVQERNSYA  2340
VSVWKRVKAK LEGRDVDPNR RMSVAEQVDY VIKEATNLDN LAQLYEGWTA WV          2392
```

FIG. 2A

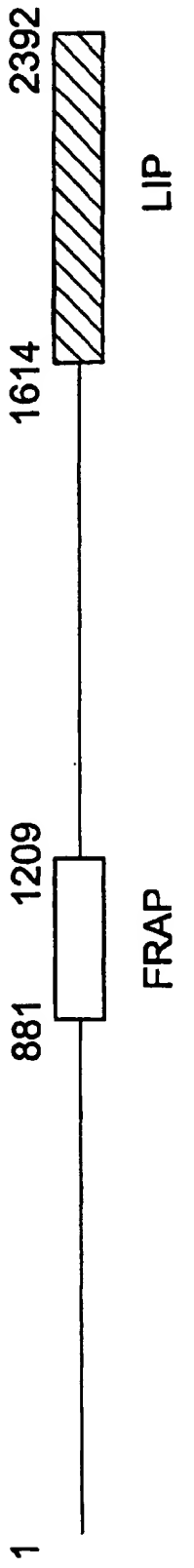

FIG. 2B

```
BLIB    821 LRLEEISPWLAAMTNTEIALPGEVSARDTVT-IHSVGGTITILPTKTKPKKLLFLGSDGK
FRAP   2121 LELQYVSPKLLMCRDLELAVPGTYDPNQPIIRIQSIAPSLQVITSKQRPRKLTLMGSNGH
            * *   ** *      * * **          * *          *  * **   ** *

BLIP    880 SYPYLFKGLEDLHLDERIMQFLSIVNTMFATINRQETPRFHARHYSVTPLGTRSGLIQWV
FRAP   2181 EFVFLLKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWV
             * ** ***  *** **    ***  *              * * ** * **** **

BLIP    940 DGATPLFGLYKRWQQREAALQAQKAQDSYQTPQNPGIVPRPSELYYSKIGPALKTVGLSL
FRAP   2241 PHCDTLHALIRDYREKKK----------------ILLNIEHRIMLRMAPDYDHLTLMQ
             *  *                              *              *      *

BLIP   1000 DVSRRDWPLHVMKAVLEELMEATPPNLLAKELWSSCTTPDEWWRVTQSYARSTAVMSMVG
FRAP   2283 KVE----------VFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRTNYTRSLAVMSMVG
             *           * *     *    *** **         *      * ** *******

BLIP   1060 YIIGLGDRHLDNVLIDMTTGEVVHIDYNVCFEKGKSL-RVPEKVPFRMTQNIETALGVTG
FRAP   2332 YILGLGDRHPSNLMLDRLSGKILHIDFQDCFEVAMTREKFPEKIPFRLTRMLTNAMEVTG
            ** ******  *   *   *   ***   ***        *** *** *     *  ***

BLIP   1119 VEGVFRLSCEQVLHIMRRGRETLLTLLEAFVYDPLVDW
FRAP   2392 LDGNYRITCHTVMEVLREHKDSVMAVLEAFVYDPLLNW
              *  *  *  *    *         ********* *
```

FIG. 3A

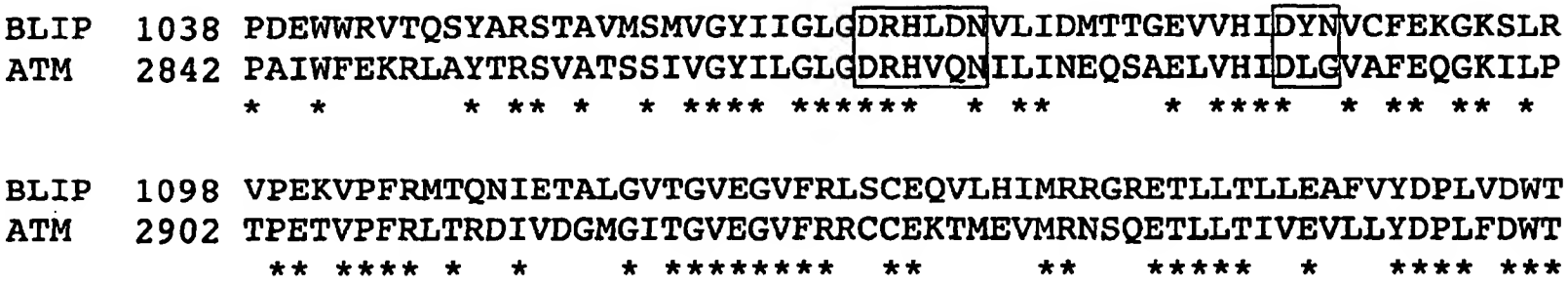

FIG. 3B

EP 1 231 264 A1

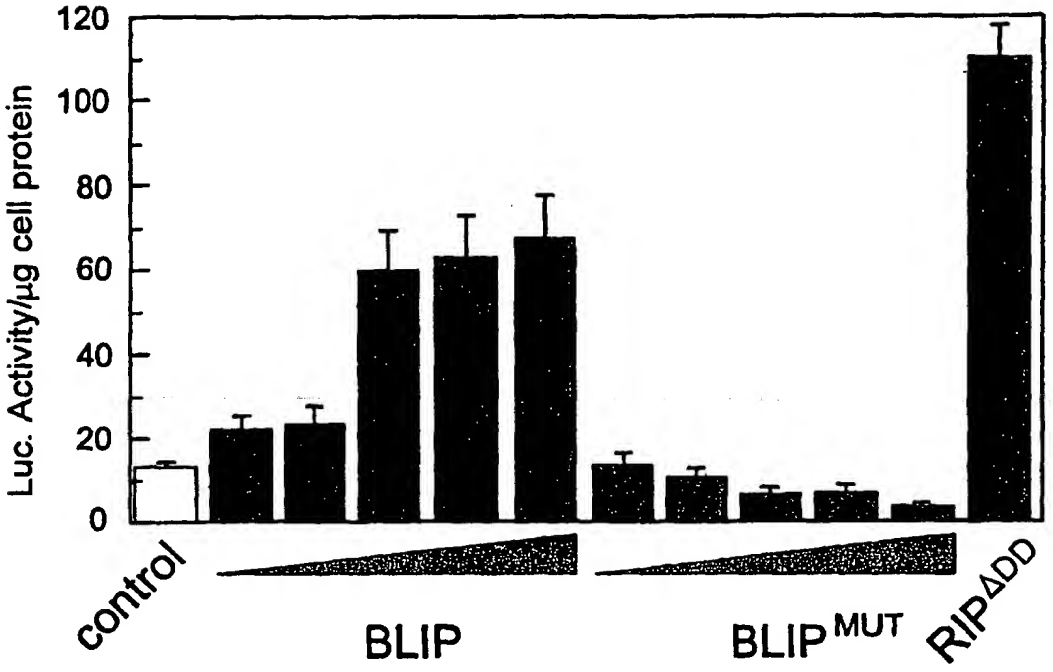
120
100
80
60
40
20
0
Luc. Activity/µg cell protein
control
BLIP
BLIP MUT
RIPΔDD

FIG. 4A

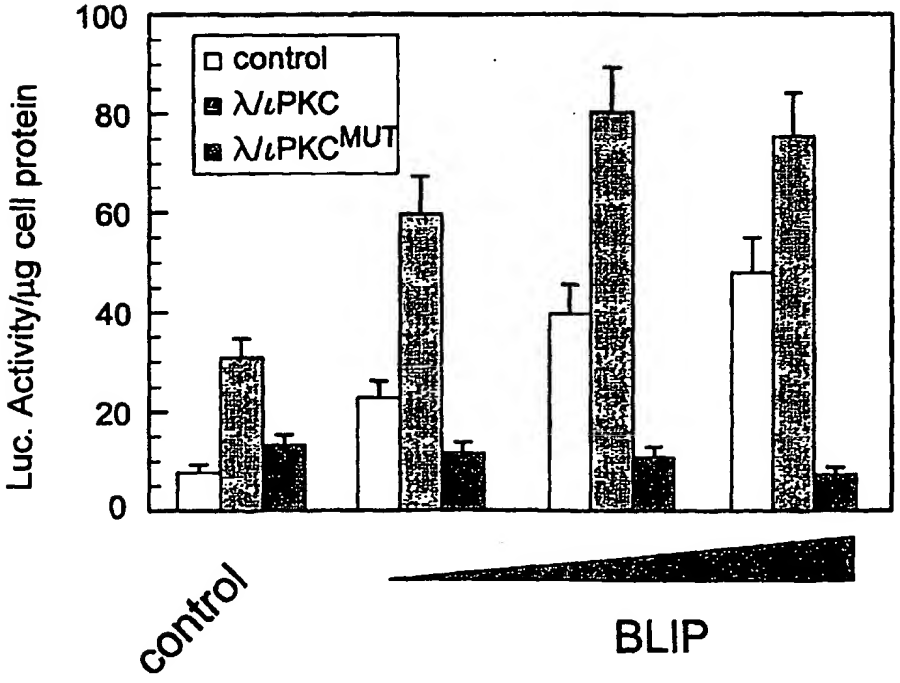
100
80
60
40
20
0
Luc. Activity/µg cell protein
control
λ/ιPKC
λ/ιPKCMUT
control
BLIP

FIG. 4B

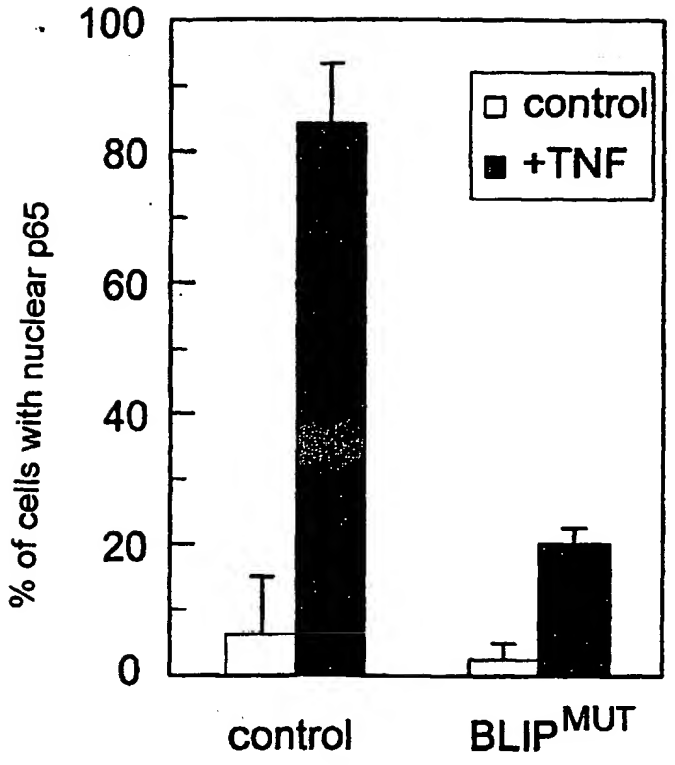
100
80
60
40
20
0
% of cells with nuclear p65
control
+TNF
control
BLIP^MUT

FIG. 5

```
AGAAAAAATA GAGTTTCACT CTGTTGCCCA GGCTGGAGTG CAGTGACGCA ATCTCGGCTC   60
ACTGCAGCCT CTGCCCTCTG AGTTCAAGTG ATTCTCCTGC CTCAGCCTCC TGAGTAGCTG  120
GGATTACAGG TGTCTGCCAC CGTGCCCAGA CATGAAAAAA CTGCTTCCTA ACATGTTAAG  180
TCCGGATCCG AGGGAACTTC AGAAATCCAT TGAAGTTCAA TTGTTAAGAA GTTCTGTTTG  240
TTTGGCAACT GCTTTAAACC CGATAGAACA AGATCAGAAG TGGCAGTCTA TAACTGAAAA  300
TGTGGTAAAG TACTTGAAGC AAACATCCCG CATCGCTATT GGACCTCTGA GACTTTCTAC  360
TTTAACAGTT TCACAGTCTT TGCCAGTTCT AAGTACCTTG CAGCTGTATT GCTCATCTGC  420
TTTGGAGAAC ACAGTTTCTA ACAGACTTTC AACAGAGGAC TGTCTTATTC CACTCTTCAG  480
TGAAGCTTTA CGTTCATGTA AACAGCATGA CGTGAGGCCA TGGATGCAGG CATTAAGGTA  540
TACTATGTAC CAGAATCAGT TGTTGGAGAA AATTAAAGAA CAAACAGTCC CAATTAGAAG  600
CCATCTCATG GAATTAGGTC TAACAGCAGC AAAATTTGCT AGAAAACGAG GGAATGTGTC  660
CCTTGCAACA AGACTGCTGG CACAGTGCAG TGAAGTTCAG CTGGGAAAGA CCACCACTGC  720
ACAGGATTTA GTCCAACATT TTAAAAAACT ATCAACCCAA GGTCAAGTGG ATGAAAAATG  780
GGGGCCCGAA CTTGATATTG AAAAAACCAA ATTGCTTTAT ACAGCAGGCC AGTCAACACA  840
TGCAATGGAA ATGTTGAGTT CTTGTGCCAT ATCTTTCTGC AAGTCTGTGA AAGCTGAATA  900
TGCAGTTGCT AAATCAATTC TGACACTGGC TAAATGGATC CAGGCAGAAT GGAAAGAGAT  960
TTCAGGACAG CTGAAACAGG TTTACAGAGC TCAGCACCAA CAGAACTTCA CAGGTCTTTC 1020
TACTTTGTCT AAAAACATAC TCACTCTAAT AGAACTGCCA TCTGTTAATA CGATGGAAGA 1080
AGAGTATCCT CGGATCGAGA GTGAATCTAC AGTGCATATT GGAGTTGGAG AACCTGACTT 1140
CATTTTGGGA CAGTTGTATC ACCTGTCTTC AGTACAGGCA CCTGAAGTAG CCAAATCTTG 1200
GGCAGCGTTG GCCAGCTGGG CTTATAGGTG GGGCAGAAAG GTGGTTGACA ATGCCAGTCA 1260
GGGAGAAGGT GTTCGTCTGC TGCCTAGAGA AAAATCTGAA GTTCAGAATC TACTTCCAGA 1320
CACTATAACT GAGGAAGAGA AAGAGAGAAT ATATGGTATT CTTGGACAGG CTGTGTGTCG 1380
GCCGGCGGGG ATTCAGGATG AAGATATAAC ACTTCAGATA ACTGAGAGTG AAGACAACGA 1440
AGAAGATGAC ATGGTTGATG TTATCTGGCG TCAGTTGATA TCAAGCTGCC CATGGCTTTC 1500
AGAACTTGAT GAAAGTGCAA CTGAAGGAGT TATTAAAGTG TGGAGGAAAG TTGTAGATAG 1560
AATATTCAGC CTGTACAAAC TCTCTTGCAG TGCATACTTT ACTTTCCTTA AACTCAACGC 1620
TGGTCAAATT CCTTTAGATG AGGATGACCC TAGGCTGCAT TTAAGTCACA GAGTGGAACA 1680
GAGCACTGAT GACATGATTG TGATGGCCAC ATTGCGCCTG CTGCGGTTGC TCGTGAAGCA 1740
CGCTGGTGAG CTTCGGCAGT ATCTGGAGCA CGGCTTGGAG ACAACACCCA CTGCACCATG 1800
GAGAGGAATT ATTCCGCAAC TTTTCTCACG CTTAAACCAC CCTGAAGTGT ATGTGCGCCA 1860
AAGTATTTGT AACCTTCTCT GCCGTGTGGC TCAAGATTCC CCACATCTCA TATTGTATCC 1920
TGCAATAGTG GGTACCATAT CGCTTAGTAG TGAATCCCAG GCTTCAGGAA ATAAATTTTC 1980
CACTGCAATT CCAACTTTAC TTGGCAATAT TCAAGGAGAA GAATTGCTGG TTTCTGAATG 2040
TGAGGGAGGA AGTCCTCCTG CATCTCAGGA TAGCAATAAG GATGAACCTA AAAGTGGATT 2100
AAATGAAGAC CAAGCCATGA TGCAGGATTG TTACAGCAAA ATTGTAGATA AGCTGTCCTC 2160
TGCAAACCCC ACCATGGTAT TACAGGTTCA GATGCTCGTG GCTGAACTGC GCAGGGTCAC 2220
TGTGCTCTGG GATGAGCTCT GGCTGGGAGT TTTGCTGCAA CAACACATGT ATGTCCTGAG 2280
ACGAATTCAG CAGCTTGAAG ATGAGGTGAA GAGAGTCCAG AACAACAACA CCTTACGCAA 2340
AGAAGAGAAA ATTGCAATCA TGAGGGAGAA GCACACAGCT TTGATGAAGC CCATCGTATT 2400
```

FIG. 6

TGCTTTGGAG CATGTGAGGA GTATCACAGC GGCTCCTGCA GAAACACCTC ATGAAAAATG 2460
GTTTCAGGAT AACTATGGTG ATGCCATTGA AAATGCCCTA GAAAAACTGA AGACTCCATT 2520
GAACCCTGCA AAGCCTGGGA GCAGCTGGAT TCCATTTAAA GAGATAATGC TAAATTTGCA 2580
ACAGAGAGCA CAGAAACGTG CAAGTTACAT CTTGCGTCTT GAAGAAATCA GTCCATGGTT 2640
GGCTGCCATG ACTAACACTG AAATTGCTCT TCCTGGGGAA GTCTCAGCCA GAGACACTGT 2700
CACAATCCAT AGTGTGGGCG GAACCATCAC AATCTTACCG ACTAAAACCA AGCCAAAGAA 2760
ACTTCTCTTT CTTGGATCAG ATGGGAAGAG CTATCCTTAT CTTTTCAAAG GACTGGAGGA 2820
TTTACATCTG GATGAGAGAA TAATGCAGTT CCTATCTATT GTGAATACCA TGTTTGCTAC 2880
AATTAATCGC CAAGAAACAC CCCGGTTCCA TGCTCGACAC TATTCTGTAA CACCACTAGG 2940
AACAAGATCA GGACTAATCC AGTGGGTAGA TGGAGCCACA CCCTTATTTG GTCTTTACAA 3000
ACGATGGCAA CAACGGGAAG CTGCCTTACA AGCACAAAAG GCCCAAGATT CCTACCAAAC 3060
TCCTCAGAAT CCTGGAATTG TACCCCGTCC TAGTGAACTT TATTACAGTA AAATTGGCCC 3120
TGCTTTGAAA ACAGTTGGGC TTAGCCTGGA TGTGTCCCGT CGGGATTGGC CTCTTCATGT 3180
AATGAAGGCA GTATTGGAAG AGTTAATGGA GGCCACACCC CCGAATCTCC TTGCCAAAGA 3240
GCTCTGGTCA TCTTGCACAA CACCTGATGA ATGGTGGAGA GTTACGCAGT CTTATGCAAG 3300
ATCTACTGCA GTCATGTCTA TGGTTGGATA CATAATTGGC CTTGGAGACA GACATCTGGA 3360
TAATGTTCTT ATAGATATGA CGACTGGAGA AGTTGTTCAC ATAGATTACA ATGTTTGCTT 3420
TGAAAAAGGT AAAAGCCTTA GAGTTCCTGA GAAAGTACCT TTTCGAATGA CACAAAACAT 3480
TGAAACAGCA CTGGGTGTAA CTGGAGTAGA AGGTGTATTT AGGCTTTCAT GTGAGCAGGT 3540
TTTACACATT ATGCGGCGTG GCAGAGAGAC CCTGCTGACG CTGCTGGAGG CCTTTGTGTA 3600
CGACCCTCTG GTGGACTGGA CAGCAGGAGG CGAGGCTGGG TTTGCTGGTG CTGTCTATGG 3660
TGGAGGTGGC CAGCAGGCCG AGAGCAAGCA GAGCAAGAGA GAGATGGAGC GAGAGATCAC 3720
CCGCAGCCTG TTTTCTTCTA GAGTAGCTGA GATTAAGGTG AACTGGTTTA AGAATAGAGA 3780
TGAGATGCTG GTTGTGCTTC CCAAGTTGGA CGGTAGCTTA GATGAATACC TAAGCTTGCA 3840
AGAGCAACTG ACAGATGTGG AAAAACTGCA GGGCAAACTA CTGGAGGAAA TAGAGTTTCT 3900
AGAAGGAGCT GAAGGGGTGG ATCATCCTTC TCATACTCTG CAACACAGGT ATTCTGAGCA 3960
CACCCAACTA CAGACTCAGC AAAGAGCTGT TCAGGAAGCA ATCCAGGTGA AGCTGAATGA 4020
ATTTGAACAA TGGATAACAC ATTATCAGGC TGCATTCAAT AATTTAGAAG CAACACAGCT 4080
TGCAAGCTTG CTTCAAGAGA TAAGCACACA AATGGACCTT GGTCCTCCAA GTTACGTGCC 4140
AGCAACAGCC TTTCTGCAGA ATGCTGGTCA GGCCCACTTG ATTAGCCAGT GCGAGCAGCT 4200
GGAGGGGGAG GTTGGTGCTC TCCTGCAGCA GAGGCGCTCC GTGCTCCGTG GCTGTCTGGA 4260
GCAACTGCAT CACTATGCAA CCGTGGCCCT GCAGTATCCG AAGGCCATAT TTCAGAAACA 4320
TCGAATTGAA CAGTGGAAGA CCTGGATGGA AGAGCTCATC TGTAACACCA CAGTAGAGCG 4380
TTGTCAAGAG CTCTATAGGA AATATGAAAT GCAATATGCT CCCCAGCCAC CCCCAACAGT 4440
GTGTCAGTTC ATCACTGCCA CTGAAATGAC CCTGCAGCGA TACGCAGCAG ACATCAACAG 4500
CAGACTTATT AGACAAGTGG AACGCTTGAA ACAGGAAGCT GTCACTGTGC CAGTTTGTGA 4560
AGATCAGTTG AAAGAAATTG AACGTTGCAT TAAAGTTTTC CTTCATGAGA ATGGAGAAGA 4620
AGGATCTTTG AGTCTAGCAA GTGTTATTAT TTCTGCCCTT TGTACCCTTA CAAGGCGTAA 4680
CCTGATGATG GAAGGTGCAG CGTCAAGTGC TGGAGAACAG CTGGTTGATC TGACTTCTCG 4740
GGATGGAGCC TGGTTCTTGG AGGAACTCTG CAGTATGAGC GGAAACGTCA CCTGCTTGGT 4800
TCAGTTACTG AAGCAGTGCC ACCTGGTGCC ACAGGACTTA GATATCCCGA ACCCCATGGA 4860

FIG. 6 CONTINUATION

AGCGTCTGAG ACAGTTCACT TAGCCAATGG AGTGTATACC TCACTTCAGG AATTGAATTC 4920
GAATTTCCGG CAAATCATAT TTCCAGAAGC ACTTCGATGT TTAATGAAAG GGGAATACAC 4980
GTTAGAAAGT ATGCTGCATG AACTGGACGG TCTTATTGAG CAGACCACCG ATGGCGTTCC 5040
CCTGCAGACT CTAGTGGAAT CTCTTCAGGC CTACTTAAGA AACGCAGCTA TGGGACTGGA 5100
AGAAGAAACA CATGCTCATT ACATCGATGT TGCCAGACTA CTACATGCTC AGTACGGTGA 5160
ATTAATCCAA CCGAGAAATG GTTCAGTTGA TGAAACACCC AAAATGTCAG CTGGCCAGAT 5220
GCTTTTGGTA GCATTCGATG GCATGTTTGC TCAAGTTGAA ACTGCTTTCA GCTTATTAGT 5280
TGAAAAGTTG AACAAGATGG AAATTCCCAT AGCTTGGCGA AAGATTGACA TCATAAGGGA 5340
AGCCAGGAGT ACTCAAGTTA ATTTTTTTGA TGATGATAAT CACCGGCAGG TGCTAGAAGA 5400
GATTTTCTTT CTAAAAAGAC TACAGACTAT TAAGGAGTTC TTCAGGCTCT GTGGTACCTT 5460
TTCTAAAACA TTGTCAGGAT CAAGTTCACT TGAAGATCAG AATACTGTGA ATGGGCCTGT 5520
ACAGATTGTC AATGTGAAAA CCCTTTTTAG AAACTCTTGT TTCAGTGAAG ACCAAATGGC 5580
CAAACCTATC AAGGCATTCA CAGCTGACTT TGTGAGGCAG CTCTTGATAG GGCTACCCAA 5640
CCAAGCCCTC GGACTCACAC TGTGCAGTTT TATCAGTGCT CTGGGTGTAG ACATCATTGC 5700
TCAAGTAGAG GCAAAGGACT TTGGTGCCGA AAGCAAAGTT TCTGTTGATG ATCTCTGTAA 5760
GAAAGCGGTG GAACATAACA TCCAGATAGG GAAGTTCTCT CAGCTGGTTA TGAACAGGGC 5820
AACTGTGTTA GCAAGTTCTT ACGACACTGC CTGGAAGAAG CATGACTTGG TGCGAAGGCT 5880
AGAAACCAGT ATTTCTTCTT GTAAGACAAG CCTGCAGCGG GTTCAGCTGC ATATTGCCAT 5940
GTTTCAGTGG CAACATGAAG ATCTACTTAT CAATAGACCA CAAGCCATGT CAGTCACACC 6000
TCCCCCACGG TCTGCTATCC TAACCAGCAT GAAAAAGAAG CTGCATACCC TGAGCCAGAT 6060
TGAAACTTCT ATTGCGACAG TTCAGGAGAA GCTAGCTGCA CTTGAATCAA GTATTGAACA 6120
GCGACTCAAG TGGGCAGGTG GTGCCAACCC TGCATTGGCC CCTGTACTAC AAGATTTTGA 6180
AGCAACGATA GCTGAAAGAA GAAATCTTGT CCTTAAAGAG AGCCAAAGAG CAAGTCAGGT 6240
CACATTTCTC TGCAGCAATA TCATTCATTT TGAAAGTTTA CGAACAAGAA CTGCAGAAGC 6300
CTTAAACCTG GATGCGGCGT TATTTGAACT AATCAAGCGA TGTCAGCAGA TGTGTTCGTT 6360
TGCATCACAG TTTAACAGTT CAGTGTCTGA GTTAGAGCTT CGTTTATTAC AGAGAGTGGA 6420
CACTGGTCTT GAACATCCTA TTGGCAGCTC TGAATGGCTT TTGTCAGCAC ACAAACAGTT 6480
GACCCAGGAT ATGTCTACTC AGAGGGCAAT TCAGACAGAG AAAGAGCAGC AGATAGAAAC 6540
GGTCTGTGAA ACAATTCAGA ATCTGGTTGA TAATATAAAG ACTGTGCTCA CTGGTCATAA 6600
CCGACAGCTT GGAGATGTCA AACATCTCTT GAAAGCTATG GCTAAGGATG AAGAAGCTGC 6660
TCTGGCAGAT GGTGAAGATG TTCCCTATGA GAACAGTGTT AGGCAGTTTT TGGGTGAATA 6720
TAAATCATGG CAAGACAACA TTCAAACAGT TCTATTTACA TTAGTCCAGG CTATGGGTCA 6780
GGTTCGAAGT CAAGAACACG TTGAAATGCT CCAGGAAATC ACTCCCACCT TGAAAGAACT 6840
GAAAACACAA AGTCAGAGTA TCTATAATAA TTTAGTGAGT TTTGCATCAC CCTTAGTCAC 6900
CGATGCAACA AATGAATGTT CGAGTCCAAC GTCATCTGCT ACTTATCAGC CATCCTTCGC 6960
TGCAGCAGTC CGGAGTAACA CTGGCCAGAA GACTCAGCCT GATGTCATGT CACAGAATGC 7020
TAGAAAGCTG ATCCAGAAAA ATCTTGCTAC ATCAGCTGAT ACTCCACCAA GCACCGTTCC 7080
AGGAACTGGC AAGAGTGTTG CTTGTAGTCC TAAAAAGGCA GTCAGAGACC CTAAAACTGG 7140
GAAAGCGGTG CAAGAGAGAA ACTCCTATGC AGTGAGTGTG TGGAAGAGAG TGAAAGCCAA 7200
GTTAGAGGGC CGAGATGTTG ATCCGAATAG GAGGATGTCA GTTGCTGAAC AGGTTGACTA 7260
TGTCATTAAG GAAGCAACTA ATCTAGATAA CTTGGCTCAG CTGTATGAAG GTTGGACAGC 7320

FIG. 6 CONTINUATION

CTGGGTGTGA ATGGCAAGAC AGTAGATGAG TCTGGTTAAG CGAGGTCAGA CATCCACCAG 7380
AATCAACTCA GCCTCAGGCA TCCAAAGCCA CACCACAGTC GGTGGTGATG CAACTGGGGG 7440
CTTACTCTGA GGAAACCTAG GAAATCTCGG TGCACTAGGA AGTGAATCCC GCAGGACAGC 7500
TGCACTCAGG GATACGCCCA ACACCATGGC CTGCAACCCC AGGGTCAAGG GTGAAGGAAA 7560
GCAAGCTCAC CGCCTGAACA CGGAGATTGT CTTTCTGCCA CAGAACAGCA GCAGACGTGT 7620
CGGGAGGTTA GCTGCGGAAA GAAATCGGGA TGCCGCGGAG CACAGAGTGA TTTGGAACTC 7680
CATTCCACCT GACCCTGTGT GTACAATCCA GGAAAAAAAC AAACCCCACT CAGAAACAGA 7740
GAAAACTGGG GTCGCGAAGA AATCACAGCC AAGGAAGATT TGATGCATTC AGATTCTCGT 7800
GTAACACTTG TTGCTTGGCA ACAGTACTGG TTGGGTTGAC CAGTAAGTAG AAAAAGGCTA 7860
AAGGCTATGC GATATGAATT TCAGAAATGG ACTGAAAATG GAGAGCTATG TAACAGATAC 7920
ACTACAGTAG AAGAACTTAC TTCTGAAATG AAGGGAAAAA AACCACCCCA TCGTTCCCTA 7980
CTCCTCCCCA CCACTTACCC GTTCCCCCTT TACCTAATCT AGTAGATTAG CCATCTTTCA 8040
AATTCACTTT TATTTCAGTC CTTATATTTC ATATACTTCC GTCTCGATGC TGTTAACAAC 8100
TTCTGATAAC ATGGAAAATT CAAGGATTGT TTAAAGGTCT GATGATCACA CACAAAATGT 8160
AATTCCGGTT ATTTAAGTCA TTTCTGTGAT TCTATCATGT ACAGTTTCCA GAATTGTCAC 8220
TGTGCATTCA AAAGTAATGA ATCTAACAGA CATTTGATTT AATGTACACT CCCTTTTGCT 8280
TATAGTGTGC ATTTTTTTTG GAGGTCATTC AAATTTTCCC TCTTCTGTGA TAGCTGTAGT 8340
TTCTTTCATA GAAAGTAGCT AATCCAGTGT AATCTTTTAC CTTTTTAAAA ACCAAGATAG 8400
AGTATCTATT AGAGTTTTAC ATTGTTGATG ATAGATTAAC AATAAAGTGA TGTTCTGGTG 8460
GAGGTAGACT GAAATTTTTT TAATTCATGT TTTTCATTTG ATACTTTTAA TTTACACTTA 8520
GTAAATTAAA AGTTGTTTAA TTTACTTGGC ATTTTAGGAC ATGTACATGA AACAGTGAAA 8580
ATGAGATCCA CCAACATCTT TTATTAAGTT CAGTTATTAG TCTGTGAAGT GCTTTACTTT 8640
TTGCACAATT TTAATAGCTT GCTATTCAGT AATACATTAT AGTGAATTCA TGATCAAGGT 8700
TTCCTTAAAT TTAGCATTGC ATTTCAGTAC TGACTGTGTA AGCTAAATTG CTGATCCAAA 8760
ATAAAAACCC AGACTAGAAT AGGGTTCTTA AAATCAAGTA TCAATACAAA ATAGAACACA 8820
ATTAAAATCT TAATTGTTGG CTGGGCACAG TGGCTCACGC CTGTAATCCC AGCACTTTGG 8880
GAGGCCGAGG CGGGCGGATC ATGAGGTTAG GAGAGCGAGA CCATCCTGGC GAACACGGTG 8940
AAACCCCGTC TTTACTAAAA TACAAAAAAA ATTAGCCGGG TGTGGTGGCG GGCGCCTGTA 9000
GTCCCAGCTA CTCGGGAGGC TGAGGCAGGA GAATGGCGTG AACCCAGGAG GCGGAGCTTG 9060
CAGTGAGCCG AGATTGTGCC ACTGCACTCC AGCCTGGGCA ACAGAGCTAG ACTCTGTGTC 9120
AAAAATAAAT GACTAGAT 9138

FIG. 6 CONTINUATION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ES 00/00308

A. CLASSIFICATION OF SUBJECT MATTER [6]:

IPC7 : C12N 9/12, 15/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 : C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STRAND, WPI, EPODOC, HCAPLUS, MEDLINE, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARIA T. DIAZ-MECO ET AL. "Lambda -Interactin Protein, a Novel Protein That Specifically Interacts with the Zinc Finger Domain of the Atypical Protein Kinase C Isotype 9/7 and Stimulates Its Kinase activity in Vitro and Vivo". Molecular and Cellular Biology, Jan. 1996, pages 105-114. Cited in the application.	5, 9-15
A		1-8, 16-26
A	LABORATORIO GLAXO WELLCOME-CSIC DE BIOLOGIA MOLECULAR Y CELULAR, "Localization of atypical Protein Kinase C into Lysosome- Targeted Endosomes throug Interaction with p62", Molecular and Cellular Biology, May 1998, pages 3069-3080. Cited in the application	1-26
A	BROWNE . J. et al. "A Mamalian Protein Targeted by G1-arresting Rapamycin-Receptor Complex. Nature" (1994) 369: 756-758.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2000 (20.12.00)	08 January 2001 (08.01.01)
Name and mailing address of the ISA/ European Patent Office Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ ES 00/00308

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims 24 and 25 relate to an object which this Authority is not obliged to search, namely: although they relate to a method for treatment of the human or animal body, the search was carried out and was based on the cited effects of the compounds.

2. ☐ Claims 5, 9-15 relate to elements in the international application which do not comply with established requirements in such a way that a meaningful search cannot be carried, more precisely: although seq. ID 16 is not included as such in the description nor in the section dealing with the lists of sequences, it was deduced from seq. ID 14.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)